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**Defining the burden of morbidity
and mortality due to invasive
Staphylococcus aureus disease
and the impact of drug resistance
in Thailand**

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BA (Hons), MA (Cantab), MBBS (Hons), MRCP**

**Doctor of Philosophy Thesis
Life and Biomolecular Sciences
The Open University**

**Wellcome Trust – Mahidol University – Oxford Tropical Medicine
Research Unit, Thailand**

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Publications arising from this thesis

EK Nickerson, V Wuthiekanun, NP Day, W Chaowagul and SJ Peacock. Methicillin-resistant *Staphylococcus aureus* in rural Asia. *Lancet Infect Dis* 2006;6(2):70-71.

EK Nickerson, TE West, NP Day and SJ Peacock. *Staphylococcus aureus* disease and drug resistance in resource-limited countries in south and east Asia. *Lancet Infect Dis* 2009;9(2):130-135.

EK Nickerson, M Hongsuwan, D Limmathurotsakul, V Wuthiekanun, KR Shah, P Srisamang, W Mahavanakul, T Wacharaprechasgul, VG Fowler, TE West, N Teerawatanasuk, H Becher, NJ White, W Chierakul, NP Day and SJ Peacock. *Staphylococcus aureus* bacteraemia in a tropical setting: patient outcome and impact of antibiotic resistance. *PLoS One* 2009;4(1):e4308

EK Nickerson, V Wuthiekanun, G Wongsuvan, D Limmathurotsakul, P Srisamang, W Mahavanakul, J Thaipadungpanit, KR Shah, A Arayawichanont, P Amornchai, A Thanwisai, NP Day and SJ Peacock. Factors predicting and reducing mortality in patients with invasive *Staphylococcus aureus* disease in a developing country. *PLoS One* 2009;4(8):e6512

Abstract

A retrospective study of *Staphylococcus aureus* bacteraemia in a provincial hospital in northeast Thailand established that *S. aureus* was responsible for considerable morbidity and mortality. A prospective study of 270 patients with invasive *S. aureus* infection in the same hospital showed that the range of clinical manifestations was broad and comparable to that in developed countries. All-cause and *S. aureus*-attributable mortality rates were 26% and 20%, respectively. Multiple logistic regression analysis revealed that age, underlying cardiac disease and respiratory infection were risk factors for all-cause and *S. aureus*-attributable mortality, while abscesses and procedures for infectious source control were associated with survival. Patients infected by Panton-Valentine Leukocidin (PVL) gene-positive isolates had a strong survival advantage compared with patients infected by PVL gene-negative isolates. Evaluation of the patient sub-set with bacteraemia (n=98) demonstrated that the prevalence of endocarditis was similar to developed countries (14%), but that the all-cause and attributable mortality rates were considerably higher (52% and 44%, respectively). Methicillin-resistant *S. aureus* (MRSA) was responsible for 28% of bacteraemias, all of which were healthcare-associated. MRSA were multidrug resistant, and the proportion of MRSA-infected patients with bacteraemia prescribed an effective antimicrobial prior to culture results becoming available was low (15%). A subset of 73 patients with severe sepsis due to *S. aureus* bacteraemia was examined to consider why death occurred and how, by drawing on the 'Surviving Sepsis Campaign' guidelines, these might be prevented. Key findings were that the core elements of the guidelines were within the current resource capability of the hospital. A prospective study of rates of MRSA carriage and acquisition in 2 intensive care units demonstrated high rates of transmission and low compliance with hand-washing. In summary, *S. aureus* disease is an important pathogen in provincial Thailand and is probably responsible for a significant burden of disease across resource-restricted Asia.

Chapter 1. Introduction

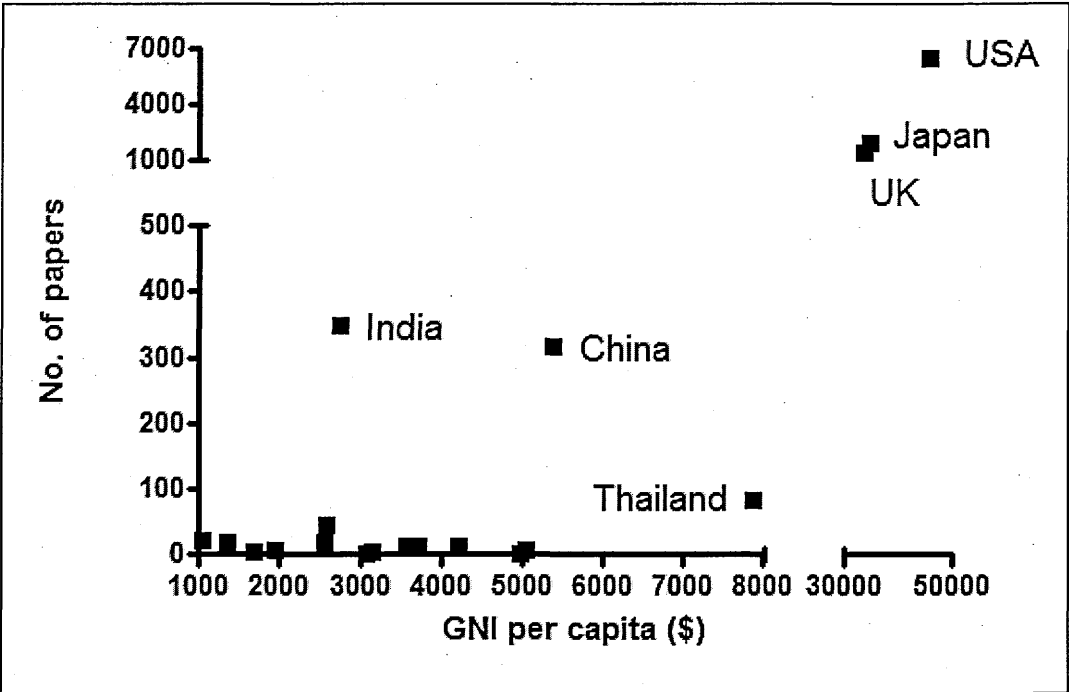
1.1 *Staphylococcus aureus* disease

Staphylococcus aureus is a Gram-positive bacterium that can both colonise and infect animals and humans. It is well established as a major cause of community-acquired and nosocomial infection in developed countries.¹⁻⁶ A review of bacteraemias in England and Wales between 1990 and 1998 found that *S. aureus* was the second most common cause annually after *Escherichia coli* and one of the top five causes in every age group.³ In the USA, *S. aureus* was the leading cause of hospital-acquired pneumonia and surgical site infections, and the second most common cause of nosocomial bacteraemia (after coagulase-negative staphylococci) during the period 1990 through 1992 according to data from the National Nosocomial Infections Surveillance system of the Centers for Disease Control and Prevention.⁷ In sharp contrast to wealthy developed countries, *S. aureus* disease ranks low on the public health agenda of resource-restricted countries in the developing world. *S. aureus* disease in these settings is perceived as trivial in terms of morbidity and mortality compared with other infectious diseases such as malaria, tuberculosis, HIV and pneumococcal infections. On closer inspection, however, the neglected status of *S. aureus* as a developing world pathogen does not equate with low rates of disease. This view is based on an evaluation of the growing body of literature on *S. aureus* disease originating from low income and lower middle income countries in South and East Asia comprised in large part of retrospective studies or case reports, but including several recent prospective studies.⁸⁻¹¹

All of the work described in this thesis was focused on and performed in Thailand. In order to place this work in context, the literature reviewed covers the different features of *S. aureus* disease and carriage in resource-restricted South and East Asia, and is compared and contrasted with developed countries. Asia is the largest and most populous continent comprised of a patchwork of developed and developing countries in close proximity. Study of this region, therefore, serves as a useful proxy for understanding staphylococcal disease worldwide. Countries included in this literature review as resource-

restricted are those classified by the World Bank as being low income or lower middle income on the basis of gross national income (GNI) per capita.¹² The countries included are Afghanistan, Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Lao People's Democratic Republic (Lao PDR), Maldives, Mongolia, Myanmar (Burma), Nepal, North Korea, Pakistan, Philippines, Sri Lanka, Thailand, Timor-Leste and Vietnam. Together, the 3.37 billion inhabitants of these countries comprise more than half of the world's population and account for over 70% of the 4.73 billion people worldwide who live in resource-restricted areas.¹³ The disparity in emphasis on *S. aureus* between these resource-restricted countries in South and East Asia and developed countries such as the UK, USA and Japan is highlighted in Figure 1.1.

Figure 1.1: Comparison of the relative volume of published papers on *S. aureus* from resource-restricted countries in South and East Asia compared with Japan, the UK and USA.



Each square represents one country. The countries clustered close to the x axis are Bangladesh, Bhutan, Cambodia, Indonesia, Lao People’s Democratic Republic (Lao PDR), Maldives, Mongolia, Nepal, Pakistan, Philippines, Sri Lanka, Timor-Leste and Vietnam. Since Afghanistan, Myanmar (Burma) and North Korea do not have gross national income (GNI) per capita data available on the World Bank website, they are not included on the graph; however, they all have 5 or fewer papers published on *S. aureus* disease.

1.1.1 Epidemiology

Amongst the papers published on *S. aureus* disease from South and East Asia there is a notable predilection for the very young, neonates in particular. In the first reported blood culture study from Lao PDR, *S. aureus* was the most common cause of bacteraemia in children under one year of age.⁸ Examination of additional studies of *S. aureus* disease that included patients of all ages¹⁴⁻²⁵ revealed that several confirmed this observation.^{14,15,20} A large number of studies described infection solely in neonates,²⁶⁻⁴⁸ with some focussed on neonatal intensive care units,^{31,32,34} or special care baby units.^{40,44,45} These observations contrast with age-specific rates of invasive *S. aureus* disease in affluent nations, where invasive *S. aureus* disease has been reported to either increase with age^{49,50} or to reach maximal incidence at the extremes of age.⁵¹⁻⁵³ However, it might be that *S. aureus* infection also occurs at a higher frequency in the elderly in resource-poor Asia, and that a possible reporting bias or age-related differences in the pattern of hospital referral or admission have resulted in under-representation of disease in this group. Alternatively, there may be increased availability of microbiological facilities in hospitals with neonatal care units or greater use of these facilities by neonatal care units, resulting in over-representation of neonates. Accurately defining the epidemiology of staphylococcal infection in many developing countries is hampered by significant variability in medical care, inadequate diagnostic microbiology facilities and lack of published studies.

1.1.2 Risk factors for disease

Risk factors for *S. aureus* disease in wealthy developed countries include nasal carriage,^{1,54-56} presence of invasive devices,^{1,2,50,55,57} surgical procedures^{1,55,57} and for methicillin-resistant *S. aureus* (MRSA), hospitalisation^{2,49,55} and antibiotic exposure.^{1,55,58} In resource-restricted South and East Asia, medical devices⁵⁹⁻⁶¹ or procedures⁶²⁻⁶⁴ and preceding antibiotic exposure⁶⁵⁻⁶⁸ have been identified as risk factors for the development of nosocomial *S. aureus* infection, but hospitalisation as a risk factor for MRSA colonisation and infection has been the subject of very few published studies.^{16,65}

Available data including some limited molecular typing link MRSA with the hospital setting. Risk factors relating to *S. aureus* infection in neonates are poorly defined. Practices relating to umbilical stump care and vaccination may be involved, and a lack of infection control procedures and associated nosocomial infection have been described.^{43,47}

1.1.3 Clinical manifestations

S. aureus is known to give rise to a wide range of superficial skin infections and deep infections affecting bones, joints, heart valves and solid organs in developed countries.^{1,69} Although many studies from South and East Asia describe a single type or category of clinical presentation, the collation of this published work indicates that the range of clinical manifestations of *S. aureus* infection reported from resource-restricted Asia is as broad as that observed in other settings. These include bacteraemia,^{8,11,14,15,25,70-88} endocarditis,^{75,89-97} meningitis and brain abscess,^{20,23,33,63,70,98-101} pneumonia,^{59,86,102-123} empyema,^{104,124} septic arthritis^{30,125-127} and osteomyelitis,^{30,127-129} abscesses of solid organs,¹³⁰ pyomyositis¹³¹⁻¹⁴⁰ and skin and soft tissue infections.^{9,10,16,29,32,62,111,115,117,141-155} The overwhelming majority of published studies involving neonates relate to bacteraemia,^{26-28,31,33-35,37-41} including both early onset sepsis (variably defined as either within 48 hours, within 72 hours²⁷ or the first week of life),^{28,31} and late onset sepsis (more than 48 hours,^{26,34} more than 72 hours²⁷ or more than 1 week).^{28,31} Bacteraemia is also the most common presentation of serious infection reported in older children,^{8,11,14,15,25,71-76} although lower respiratory tract infections¹⁰²⁻¹⁰⁹ (including empyema)¹²⁴ and endocarditis⁸⁹⁻⁹³ are also described. Despite the textbook focus on pyomyositis as the “tropical” form of *S. aureus* disease,¹⁵⁶⁻¹⁵⁸ there are just 10 studies describing pyomyositis in the literature from our target countries.¹³¹⁻¹⁴⁰ Thus, *S. aureus* infection in resource-restricted Asia is not only common but frequently presents as invasive disease.

Once *S. aureus* bacteria are in the bloodstream they can disseminate throughout the body, which results in metastatic foci of infection at sites distant to the original source or point of entry.¹ It can be very hard clinically to detect those patients with metastatic

infection. For example, Fowler et al demonstrated that amongst patients with *S. aureus* bacteraemia, predisposing heart disease and clinical findings did not differentiate those with and without infective endocarditis and that transoesophageal echocardiography was required.¹⁵⁹ A further clinical challenge in those identified with complications is to then determine the course of infection in terms of primary site compared with metastatic foci. When an intravenous device is the source of infection for *S. aureus* bacteraemia, subsequent sites of infection identified are more likely to have seeded from the site of the initial intravenous device infection. As there is an on-going trend for an increasing proportion of *S. aureus* bacteraemias to result from intravenous devices,^{2,160} ascertaining clinical, imaging or laboratory tests to assist in identifying patients at greatest risk of metastatic infection becomes more pertinent. A study from the USA demonstrated that a scoring system based on the presence or absence of 4 risk factors (community acquisition, skin examination findings suggesting acute systemic infection, persistent fever at 72 hours, and positive follow-up blood culture results at 48 to 96 hours) accurately identified complicated *S. aureus* bacteraemia, with a positive follow-up blood culture being the strongest predictor.¹⁶¹ In intravenous catheter-related bloodstream infection, persistent fever and/ or repeated positive blood cultures after 3 days following intravenous catheter removal has been shown to predict complicated *S. aureus* bacteraemia.¹⁶²

1.1.4 Mortality

It is not possible to determine the death rate for serious *S. aureus* infection from the studies published from resource-restricted Asia. The majority considered all-cause bacterial sepsis in which *S. aureus* disease represented a small subset and organism-specific outcomes were not detailed, although several studies did comment that infection with *S. aureus* carried an increased risk of death compared with other bacterial pathogens.^{91,113,163}

1.1.5 Drug resistance

Signalled by the acquisition of penicillin resistance in the 1940s,¹⁶⁴ strains of *S. aureus* have since acquired resistance to methicillin (often in association with resistance to other antimicrobial groups including macrolides, aminoglycosides and quinolones),^{165,166} and more recently intermediate, followed by full resistance to glycopeptides.^{167,168} Methicillin resistance is acquired by insertion of the staphylococcal cassette chromosome *mec* (SCC*mec*) element, which carries the gene encoding an altered penicillin binding protein. SCC*mec* elements have been acquired on multiple occasions by biologically fit *S. aureus* clones circulating in the community.¹⁶⁹⁻¹⁷¹ Penicillin resistance is now virtually ubiquitous and methicillin resistance is geographically variable but is approaching 50% for invasive isolates in many areas of Europe, America and Australia.¹⁷²⁻¹⁷⁴ The proportion of *S. aureus* blood culture isolates that are methicillin-resistant currently reaches 70% in affluent Asian countries such as Japan¹⁷⁵ and South Korea,¹⁷⁶ but rates of MRSA infection in less affluent Asian countries are generally poorly defined. Despite the perceived importance of MRSA and the large volume of research devoted to its study across the developed world, almost nothing is known about the emergence and transmission of MRSA in resource-poor regions of the world. Large-scale surveillance systems in Asia such as the SENTRY Antimicrobial Surveillance Program and ANSORP (Asian Network for Surveillance of Resistant Pathogens) focus for the large part on the more affluent countries,¹⁷⁷⁻¹⁷⁹ and isolate collections are inevitably biased towards areas that contain diagnostic microbiology facilities. Most studies describing MRSA rates involve sub-groups of patients, especially high-risk patients such as those with burns, from which meaningful conclusions on MRSA prevalence cannot be drawn. However, multi-centre MRSA surveillance data from China and India suggest that MRSA accounts for a considerable burden of disease in these countries.¹⁸⁰⁻¹⁸² Additionally, data published from the National Staphylococcal Phage Typing Centre in India shows that MRSA rates rose annually during the 1990s.¹⁸³

Given the patchwork nature of the available data on MRSA, the prevalence of MRSA infection across large areas of resource-restricted Asia is unknown but may be

considerable, if extrapolations are made from the information that is published. The dissemination of MRSA across low-income regions would have major implications for the antibiotic treatment of, and outcome from serious *S. aureus* disease. MRSA infection is likely to go unnoticed as a consequence of the widespread lack of diagnostic microbiology facilities, leading to the probability of ineffective antibiotic prescribing and an inevitable increase in the rate of poor outcome.¹⁸⁴⁻¹⁸⁶ As elsewhere in the world, serious MRSA infection in Asia requires treatment with glycopeptides, but these relatively expensive antibiotics are of limited availability across low-income settings and require administration by intravenous injection. Furthermore, optimal glycopeptide dosing requires monitoring of serum drug levels, a capability which is usually lacking.

1.1.5.1 Community-acquired MRSA

MRSA was initially a problem limited to hospital-adapted isolates but has now extended to arise de novo in community isolates.¹⁸⁷⁻¹⁹⁰ Community-acquired (CA-) MRSA carry novel *SCCmec* elements.^{191,192} This has spawned a plethora of research in the developed world and initiated much discussion over the challenges posed for antibiotic prescribing.^{193,194} Classification of CA-MRSA varies in the literature but at its most basic requires that there has been no contact with healthcare facilities, usually for at least 1 year. There are very few studies from resource-restricted Asia describing MRSA infection in the absence of contact with healthcare facilities.^{16,124,144} In these reports, putative CA-MRSA (as defined by lack of hospital contact) accounted for 3-11% of *S. aureus* infections: 3 CA-MRSA from 88 *S. aureus* isolates associated with empyema thoracis (3%);¹²⁴ 6 CA-MRSA from 116 *S. aureus* isolated from varied pus samples (5%);¹⁴⁴ and 22 CA-MRSA from 202 *S. aureus* pyoderma (11%).¹⁶ This suggests that CA-MRSA is beginning to emerge but its true prevalence is not known. These studies did not apply the molecular tools commonly used to characterise MRSA, such as *SCCmec* typing (which is usually type IV or V for CA-MRSA), pulsed field gel electrophoresis (PFGE) or multilocus sequence typing (MLST).

Over-the-counter antibiotics, such as penicillins (including cloxacillin), cephalosporins, tetracyclines, quinolones and trimethoprim-sulfamethoxazole, are readily

available from pharmacists across much of Asia and these are frequently self-administered for inappropriate indications, and taken for irregular durations.¹⁹⁵⁻¹⁹⁷ The cost of ten tablets of cloxacillin purchased over the counter from a pharmacy in provincial Thailand in 2007 was around £1 (\$1.80 at 2007 exchange rates), and the cost of 10 tablets of amoxicillin, doxycycline or ciprofloxacin was around 85 pence (\$1.50) (personal experience). This uncontrolled antimicrobial use is likely to fuel the emergence of CA-MRSA as well as drug resistance in a broad range of other human pathogens. Sub-standard antibiotics are commonly reported from resource-restricted Asia,¹⁹⁸ the presence of which could further drive resistance.

1.1.5.2 Vancomycin resistance

Reduced susceptibility to vancomycin was first described from Japan.^{168,199} Since then full resistance to vancomycin has been reported from the USA²⁰⁰⁻²⁰² and Tehran,²⁰³ and intermediate resistance from Belgium,²⁰⁴ Brazil,²⁰⁵ France,²⁰⁶ Korea,²⁰⁷ Portugal,²⁰⁸ Scotland²⁰⁹ and South Africa.²¹⁰ Given that vancomycin is the mainstay of treatment for MRSA, the emergence of glycopeptide resistance is of great concern, although it is currently uncommon in developed countries.²¹¹⁻²¹³ Amongst the resource-restricted countries of Asia, full resistance to vancomycin has been reported from India in an evaluation of 783 clinical *S. aureus* isolates collected between August 2002 to July 2005.²¹⁴ Two isolates were vancomycin resistant (minimum inhibitory concentration (MIC) 32 µg/ml and MIC 64 µg/ml, respectively) and 6 isolates had intermediate resistance (MIC 16 µg/ml for 2 isolates and MIC 8 µg/ml for 4 isolates) as defined by agar dilution. None of these isolates was positive by polymerase chain reaction (PCR) for *vanA*. The gene *vanA* encodes resistance to vancomycin in vancomycin-resistant *S. aureus* (VRSA) and is likely to be acquired from vancomycin-resistant enterococci (VRE). However, the reduced susceptibility seen in vancomycin-intermediate *S. aureus* (VISA) is not mediated by the *van* genes, but is associated with a marked thickening of the cell wall.²¹⁵ A further VRSA (MIC ≥64µg/ml) has been isolated from the pus specimen of an out-patient in Kolkata, India in 2005, which was positive for *vanA*.²¹⁶

Heterogeneous vancomycin-intermediate *S. aureus* (hetero-VISA) refers to an isolate with a sub-population of colonies demonstrating intermediate resistance to vancomycin. A study of isolates collected by ANSORP reported the isolation of hetero-VISA from India (5 isolates, 6.3% of all Indian MRSA tested), Thailand (2 isolates, 2.1%), Vietnam (1 isolate, 2.4%) and the Philippines (1 isolate, 3.6%).¹⁷⁹ A further 4 hetero-VISA have been reported from Thailand.^{75,217} The majority of other studies evaluated in this review used a disk diffusion method for detection of vancomycin susceptibility, an inaccurate method for the assessment of intermediate resistance.²¹² Although isolates with reduced susceptibility to vancomycin have been identified in my target countries, they remain uncommon at present.

1.1.6 Bacterial typing

S. aureus epidemiology has relied extensively in recent years on the use of MLST to define bacterial population genetic structure. Studies of MRSA including isolates from my target countries have demonstrated a predominance of sequence type (ST) 239 (SCCmec type III or IIIA) and ST 5 (SCCmec type II or IV).^{218,219} A study of 74 MRSA isolates from 12 Asian countries (5 or 6 isolates from most countries) collected between 1998 to 2003 found that most isolates from South Korea and Japan were ST 5, whereas ST 239 predominated in the other Asian countries including China, Indonesia, India, Philippines, Sri Lanka, Thailand and Vietnam.²¹⁸ A subsequent study from South Korea suggested that ST 239 had recently emerged there.²²⁰ Further studies have confirmed the predominance of ST 239 in China, India, Mongolia and Thailand.²²¹⁻²²⁵ A study conducted in China of a selected group of MRSA isolates (selected on the basis of positivity for genes encoding Pantone-Valentine Leukocidin) reported equal numbers of ST 88, ST 239 and ST 398.²²⁶ The utility of typing in a developing country setting was demonstrated by a report describing the investigation of an outbreak of MRSA infection in Vietnam that was associated with vaccination.²²⁷ *S. aureus* of an identical genotype was isolated from injection site abscesses of 4 children, and nasal and throat swabs from their vaccinator,

typing of which demonstrated that they were SCC*mec* type V (or type 5C) and ST 59, the endemic CA-MRSA clone in Taiwan.²²⁸

Non-governmental organisations and hospitals reliant on overseas volunteer healthcare staff are at risk of MRSA being introduced by one or more individuals from settings where MRSA carriage occurs. A study from Lao PDR reporting the first isolation of MRSA in the country highlighted this potential problem. Molecular typing demonstrated that the SCC*mec* type of the isolate in question matched the type carried by CA-MRSA isolates in Japan.¹¹⁷ The hospital from which it was isolated received many Japanese exchange staff, raising the possibility of international spread by medical staff. This highlights the importance of several approaches in resource-restricted settings to identify the emergence of MRSA and understand routes of MRSA transmission, including the screening of healthcare workers prior to arrival, ongoing vigilance, and the judicious use of molecular techniques. It is also likely that overseas staff can become colonised with MRSA during work in low-income countries and return to hospitals in developed countries with these isolates.

1.1.7 Staphylococcal toxins

S. aureus can produce a number of different toxins that give rise to specific diseases.¹ Enterotoxins (staphylococcal enterotoxins A to E) can result in the development of gastroenteritis and toxic shock syndrome toxin 1 (TSST-1) causes toxic shock syndrome. The exfoliative toxins, epidermolytic toxins A and B, induce separation of the skin layers to produce scalded skin syndrome. Although a rare condition, toxic shock syndrome became widely known in the early 1980s when an increase in the number of cases was related to menstruation and the use of hyper-absorbent tampons in countries where tampon use is common, particularly North America and western Europe.²²⁹⁻²³² These toxin-induced manifestations of *S. aureus* disease are also reported from resource-restricted countries in South and East Asia. Gastroenteritis arising from staphylococcal enterotoxins has been reported from India,^{24,233-236} the Philippines²³⁷ and Thailand²³⁸ and staphylococcal enterotoxins found in a variety of clinical samples in Sri Lanka.²³⁹

Published reports of toxic shock syndrome from my target countries are all non-menstrual and include paediatric cases,²⁴⁰ following vaccination²⁴¹ and pyogenic skin infections in India,^{242,243} and vaginal infection in China.²⁴⁴ A study of scalded skin syndrome has been published from China,²⁴⁵ as have case reports from India²⁴⁶ and Thailand.²⁴⁷

1.1.7.1 Panton-Valentine Leukocidin

Panton-Valentine Leukocidin (PVL) is a bicomponent leukocytotoxin and putative virulence factor. Since the first clinical study describing an association between PVL and necrotising pneumonia in young adults was published,²⁴⁸ interest in PVL as a virulence determinant for human infection has grown at a staggering speed, fuelled in part by the finding that many emergent community-acquired MRSA isolates are PVL gene-positive.²⁴⁹⁻²⁵¹ The evidence for PVL as a virulence determinant in animal models is mixed and controversy abounds. Some studies are supportive,²⁵²⁻²⁵⁵ whilst others convincingly cast doubt on PVL being a virulence determinant.²⁵⁶⁻²⁵⁹ The leukolytic effect and hence lethality of PVL on mouse neutrophils has been shown to be less than that on human neutrophils,^{260,261} which calls into question the results from mouse-based disease models for PVL pathogenesis. Additionally, despite finding a 10-fold variation in the amount of PVL produced by disease-causing isolates, there was no correlation between quantity of PVL and disease severity.²⁶² Recent studies have elucidated that pathogenesis previously ascribed to PVL may in fact relate to other toxins, such as alpha-haemolysin²⁵⁹ and novel phenol-soluble modulin peptides,²⁶³ or disruption of a global gene regulator of *S. aureus* that controls the expression of multiple virulence determinants.²⁶⁴

In the developed world carriage of the genes encoding PVL is associated with skin and soft tissue infections²⁶⁵⁻²⁶⁹ as well as with more serious infections such as severe necrotising pneumonia.^{248,266,270-272} The clinical studies on PVL often describe selected sub-groups of patients,²⁴⁸ case reports²⁷⁰⁻²⁷³ or *S. aureus* isolates sent to a reference laboratory^{266,274} creating a disproportionate emphasis on the severe end of the disease spectrum, namely necrotising pneumonia. PVL gene-positive *S. aureus* in resource-restricted countries in South and East Asia have also been associated with skin and soft

tissue infections,^{226,227,275,276} together with a single reported case of fatal necrotising pneumonia.²⁷⁷ Data on the proportion of *S. aureus* isolates positive for PVL in South and East Asia is sparse and only available for a small number of countries. PVL has been detected in 7.2% to 13.3% of disease-associated *S. aureus* isolates in China^{226,278-280} and PVL positivity of methicillin-sensitive *S. aureus* (MSSA) carriage isolates in Indonesia was reported as 10.6%.²⁸¹

Interest in PVL as a virulence determinant has prompted the development of rapid tests for the earlier detection of the genes encoding PVL. At the time of writing there have been 2 multiplex PCR tests created for this purpose^{282,283} and proof of concept for a method involving matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry.²⁸⁴

1.1.8 Management of *S. aureus* disease

The management of *S. aureus* disease requires consideration of antibiotic therapy, infectious source control and general supportive care for sepsis tailored to the specific disease manifestation and its severity. Each of these aspects of management is discussed in turn.

1.1.8.1 Antibiotic therapy

Antibiotic therapy is the mainstay of treatment for *S. aureus* disease, with the route of administration (topical, oral or intravenous) reflecting the severity of infection. For MSSA infections, penicillinase-resistant beta-lactams are the drug group of choice,^{1,285,286} for example flucloxacillin, cloxacillin and nafcillin, depending on the drug supplied in different countries. Patients with a penicillin allergy should be treated with a non-beta-lactam antibiotic, such as erythromycin, or a cephalosporin if the allergy is minor (e.g. skin rash) since up to 10% of patients with a penicillin allergy have a cross-reaction with the cephalosporin group of antibiotics.^{287,288} Since penicillin resistance exceeds 95% worldwide, penicillin can only be used for patients infected with an isolate proven to be sensitive with *in vitro* susceptibility testing. First and third generation cephalosporins (e.g.

cefazolin and ceftriaxone, respectively) and carbapenems (e.g. meropenem) are also effective for MSSA infections, but first generation cephalosporins may not be as effective for severe infections such as endocarditis.²⁸⁵ In serious invasive MRSA infections, such as bacteraemia or pneumonia, vancomycin is the first line drug of choice.^{1,285,286} However, there are concerns with vancomycin use including the development of tolerance and resistance, poor tissue penetration and slow bactericidal activity, which is leading to increased use of newer alternative antibiotics.²⁸⁹ The newer anti-MRSA antibiotics include linezolid, daptomycin, tigecycline, dalbavancin, ceftobiprole and iclaprim,²⁹⁰⁻²⁹³ but these are expensive. For MRSA infections that are amenable to oral antibiotic therapy, such as skin and soft tissue infections, the choice of lincosamines (e.g. erythromycin, clindamycin), fluoroquinolones (e.g. ciprofloxacin), tetracyclines (e.g. doxycycline), trimethoprim-sulphamethoxazole or rifampicin and fusidic acid in combination depends on local susceptibility patterns for MRSA infections. In Thailand, a wide range of standard antibiotics are readily available, including over-the-counter in pharmacies; however, the newer anti-MRSA antibiotics are not obtainable (personal experience). Vancomycin is prescribed in Thailand but the facilities to monitor drug levels to lessen the risk of toxicity are limited and not available in most hospitals.

Delays in receiving effective antibiotic therapy are known to have a deleterious effect on outcome from *S. aureus* bacteraemia in developed countries.¹⁸⁴⁻¹⁸⁶ For appropriate antibiotic therapy to be timely, the choice of empirical antibiotics needs to predict the most likely bacterial causative agents based on local disease patterns and susceptibility profiles. Therapy can then be narrowed once culture results are available. Where background rates of MRSA are high, vancomycin is used for surgical prophylaxis and as empiric therapy for suspected *S. aureus* infection in order to prevent the inadvertent treatment of MRSA infection with cloxacillin, which is associated with a poor outcome. The down side is that a high rate of vancomycin usage drives the emergence of resistance and increases drug costs. Studies specifically addressing the timing of antibiotic therapy for *S. aureus* infection have not been reported from South and East Asia.

1.1.8.2 Infectious source control

Abscess formation is a common finding in *S. aureus* disease.¹ These collections of pus can develop locally at the portal of entry or in diverse locations around the body following haematogenous spread. In general, antibiotics alone are not sufficient to resolve deep pus collections and drainage is required.²⁹⁴ A drainage procedure without effective antibiotic therapy for localised skin and soft tissue abscesses can be sufficient to achieve cure.^{268,295,296} In developed countries, the presence of an uneradicated focus of infection has been associated with a 6.7 times increase in mortality;²⁹⁷ and failure to remove an intravenous catheter in nosocomial bacteraemia resulted in a 6.5 times increase in death or relapse of infection.²⁹⁸ In developing countries in South and East Asia, there is minimal information regarding the effect on outcome resulting from the active management of pus collections in *S. aureus* disease. A study from India discusses the differing management approaches to empyema thoracis;¹²⁴ another study from India concludes that needle aspiration is an effective alternative treatment to an open drainage procedure for pyogenic liver abscesses;¹³⁰ and surgical drainage for iliopsoas abscesses is recommended as a cost-effective primary treatment in a study from Nepal.¹⁴⁰

1.1.8.3 Supportive Sepsis Care

By standard sepsis definitions,²⁹⁹⁻³⁰¹ sepsis is a systemic inflammatory response syndrome (SIRS) resulting from the presence of infection; severe sepsis is SIRS with organ dysfunction, hypoperfusion or sepsis-induced hypotension; and septic shock is SIRS with sepsis-induced hypotension despite adequate fluid resuscitation. These terms represent increasing severity along a pathological continuum; as a patient progresses from sepsis through severe sepsis to septic shock their risk of dying increases.^{302,303} Severe sepsis and septic shock are estimated to affect millions of people each year and are important causes of mortality worldwide.³⁰⁴⁻³¹⁰

Early recognition of sepsis and prompt resuscitation are paramount to achieving a successful outcome.³¹¹ Two critical components of the initial sepsis care are early fluid

resuscitation, with the first 1 to 6 hours having the greatest impact on outcome;³¹²⁻³¹⁴ and timely administration of effective antibiotic therapy, ideally within the first hour as each hour delay over the next 6 hours after that has been shown to result in a 7.6% decrease in survival.³¹⁵ Given the vascular dilatation and leakage of plasma into the extravascular space that occurs in sepsis,³¹⁶ aggressive fluid resuscitation involving large volumes is necessary, and vasopressors and inotropes may be required in addition to maintain an adequate blood pressure.³¹¹ Despite the recommendations for fluid resuscitation in the initial hours of sepsis, an audit of paediatric sepsis care in the UK showed that 62% of children received inadequate fluid resuscitation and inotropic support,³¹⁷ and an audit of adult care found that nearly half the patients did not receive an adequate fluid challenge.³¹⁸

The other aspects of supportive sepsis care depend on the specific organ dysfunction developed by the patient. Respiratory dysfunction frequently complicates sepsis and requires ventilatory support.³¹⁹⁻³²¹ However, mechanical ventilation can also lead to considerable additional morbidity, such as ventilator associated pneumonia,^{322,323} and acute lung injury if tidal volumes are too high.³²⁴ Tidal volume control can be particularly challenging in resource-restricted developing countries where less sophisticated ventilators are used that may not facilitate fine tuning of tidal volumes or control of inspiratory pressures. Renal replacement therapy plays an important role in sepsis management for the treatment of acute renal failure and acidosis. In developed countries, haemodialysis is used in the setting of acute sepsis but in resource-restricted developing countries peritoneal dialysis may be the only available option. A study from Vietnam demonstrated the superiority of haemodialysis over peritoneal dialysis for acute infection-related renal failure.³²⁵ Since critical illness-related corticosteroid insufficiency can affect patients with severe sepsis, physiological corticosteroid replacement is currently recommended in patients with vasopressor-dependent septic shock.^{326,327} The use of insulin to achieve tight glycaemic control is controversial. Tight glycaemic control reduced the mortality of cardiac surgical intensive care patients³²⁸ but only improved morbidity not mortality in a medical intensive care setting.³²⁹ A meta-analysis of intensive

insulin therapy in critically patients did find a survival benefit,³³⁰ although it should be noted there were no medical intensive care units included. Excess hypoglycaemic episodes in patients receiving intensive insulin therapy³³¹ means that this is unlikely to be suitable for developing country settings where staffing levels may be lower. Activated protein C provides a survival advantage in patients with severe sepsis and at high risk of death (more than one organ dysfunction) but there is a considerable bleeding risk^{332,333} and is too expensive for widespread use in developing countries. Additional adjunctive therapies include deep vein thrombosis prevention^{334,335} because the critically ill are at a higher risk of deep vein thrombosis³³⁶ and stress ulcer prophylaxis because patients with severe sepsis often have risk factors identified for gastrointestinal haemorrhage in the critically ill.^{337,338}

The 'Surviving Sepsis Campaign' international guidelines for the management of severe sepsis and septic shock were first published in 2004³³⁹ and then updated in 2008.³⁴⁰ These guidelines are highly influential documents that describe best practice in resource-rich settings, and represent an important milestone in the improvement of standards of clinical care in the developed world. However, the majority of deaths arising from sepsis worldwide most likely occur in developing countries because of the huge burden of bacterial infections and resource-restriction in these regions.³⁴¹ Bacterial infections are implicated as the direct cause of death from lower respiratory infections, meningitis, and a range of other infections³⁴²⁻³⁴⁵ and also complicate other common diseases such as malaria, HIV/AIDS and diabetes mellitus.³⁴⁶⁻³⁴⁸ Although understudied, severe sepsis is associated with extremely high mortality rates in developing countries worldwide,^{321,349,350} and in South and East Asia.³⁵¹ The facilities and therapeutics available for the medical care of patients with sepsis throughout the developing countries of South and East Asia are highly variable, and a scaled approach to management based on optimising utilisation of existing resources has been suggested which could represent a practical and flexible strategy.³⁴¹

1.2 *S. aureus* carriage

The epithelial surfaces of humans are colonised by bacteria that constitute the commensal flora, including the gastrointestinal and respiratory tracts. *S. aureus* is commonly found as part of the flora.^{1,56} Colonisation occurs very early in life: *S. aureus* has been grown from the umbilical stump and groin within a day of birth and subsequently from the nose.^{352,353} The predominant site of *S. aureus* carriage is in the anterior nasal passages,^{56,354,355} but *S. aureus* colonisation has also been reported from multiple other sites around the body including the throat, axilla, groin, rectum and in skin and soft tissue wounds in developed countries.^{56,354} Studies of *S. aureus* carriage from developed countries demonstrate that colonisation is dynamic: there are changes with increasing age³⁵⁴⁻³⁵⁸ and over time for the population.^{56,359} Children carry *S. aureus* more frequently than adults^{354,355,358}, typically with those less than 8 weeks old having the highest colonisation rates and decreasing thereafter^{354,356-358} although a normal distribution with a peak at 11 years of age has also been described;³⁶⁰ and persistent carriage gives way to intermittent carriage typically between the ages of 10 and 20 years.³⁵⁵ The decrease in *S. aureus* carriage over the last 70 years is thought to reflect improvements in personal hygiene⁵⁶ and socioeconomic status³⁶¹ and decreased family sizes.³⁶⁰ In developed countries studies of *S. aureus* colonisation have shown that about 20% (range 12–30%) of the general population are persistent *S. aureus* nasal carriers and approximately 30% are intermittent carriers (range 16–70%).^{56,359} Some of the variation between intermittent carriage and persistent carriage rates in different studies is dependent upon the length of follow-up and the definitions for determining each category.^{56,359,362,363} Additionally, colonising isolates of *S. aureus* within the nose vary over time.^{363,364} Those people who have been hospitalised or require regular out-patient care are at an increased risk of carrying MRSA rather than MSSA.^{365,366} In addition, with the worldwide emergence of community-acquired MRSA there is an increasing likelihood that people with no healthcare contact may be colonised with MRSA.^{367,368}

Currently there is a single paper with details of *S. aureus* carriage from Thailand,³⁶⁹ which was detected as part of an MRSA outbreak in a burns unit and minimal

studies focusing on colonisation with *S. aureus* from South and East Asia. These include 3 months surveillance for MRSA carriage amongst patients and staff on a surgical unit in Sri Lanka;³⁷⁰ a further study from Sri Lanka describing the prevalence of and risk factors for nasal colonisation with MRSA of those admitted to a surgical unit;⁶⁵ work determining the prevalence of MRSA amongst a random sample of children in the community in Nepal,³⁷¹ a study reporting the high prevalence of *S. aureus* colonisation, including MRSA, of burn wounds in China;³⁷² MRSA surveillance studies in India involving healthcare workers,³⁷³⁻³⁷⁵ patients³⁷⁴ and healthy volunteers³⁷⁵; and a study demonstrating the effectiveness of nasal mupirocin to eradicate *S. aureus* carriage and thereby prevent catheter exit site infections and peritonitis in peritoneal dialysis patients in India.³⁷⁶

1.2.1 Relationship of carriage to infection

The importance of carriage is that the majority of patients who develop invasive *S. aureus* disease are infected with the strain with which they are colonised.^{54,56,377-379} This has been demonstrated in a variety of clinical settings including the development of post-operative wound infections,^{380,381} following peritoneal dialysis³⁷⁹ and haemodialysis³⁷⁷ and in those with human immunodeficiency virus (HIV).^{378,382} Additionally, rates of *S. aureus* infection are higher in those who are colonised with *S. aureus* than in non-carriers.^{377,379,380,382,383} As a consequence of this close relationship between colonisation and infection, eradication of colonising *S. aureus*, principally nasal carriage, is used as a strategy to prevent subsequent infections in high risk patients and when the outcome could be devastating, for example in those requiring dialysis^{377,384,385} or undergoing major surgery,³⁸⁶⁻³⁸⁸ especially surgery involving prosthetic material.³⁸⁹

1.2.2 Patterns of *S. aureus* carriage

The highest detection of *S. aureus* carriage is in the anterior nasal passages,^{56,354,355} followed by the perineum and throat.^{354,355,390-392} Lower rates of detection are found from the gastrointestinal tract, vagina and axillae.³⁹³⁻³⁹⁶ People who are nasal carriers of *S. aureus* have higher rates of colonisation with *S. aureus* at other sites around the body

than the general population.^{56,354} Rates of carriage of *S. aureus* are higher in patients undergoing regular venepuncture, such as those requiring dialysis,^{377,379} or in patients needing regular injections, such as diabetics managed with subcutaneous insulin,³⁹⁷ or in those who inject drugs recreationally.³⁹⁸ Intravenous devices such as cannulas and cut-downs provide colonising bacteria with a route of entry to the body and bloodstream.^{56,399,400} In Intensive Care Units the increased use of invasive interventions and monitoring means that patients are more vulnerable to developing hospital-acquired infections.^{399,401-404} In hospitals where MRSA is endemic the risk of infection with MRSA increases with the length of stay.^{401,403,405} Many countries routinely swab patients in high-risk areas, such as intensive care units, in order to detect MRSA colonisation before the development of infection.⁴⁰⁶⁻⁴¹⁰ The sites chosen for sampling are very variable,^{401,405,411-415} as yet there is no consensus on best practice. Screening for MRSA is typically performed weekly.^{401,414,416} Actively screening patients at high risk of MRSA, for example those previously colonised or infected, residents of nursing homes, those admitted to a healthcare facility in the preceding 12 months, those receiving recent antibiotic therapy and patients transferred from another hospital,^{406,417-419} forms the essential foundation for infection control.⁴⁰⁶

1.3 Infection control

Infection control relates to the prevention of nosocomial transmission of multi-resistant organisms within a healthcare facility. In developed countries, infection control policies are implemented in relation to patients colonised or infected with MRSA aiming to prevent the spread of MRSA to other patients.^{406,409,410} In general a whole package of infection control measures are put in place together.^{409,420,421} There are universal infection control measures such as hand hygiene, barrier nursing, isolation and cohorting and other measures specific to MRSA, for example de-colonisation.

1.3.1 Hand hygiene

Studies from developed countries have highlighted the hands of medical staff as an important route of transmission for hospital-acquired infections, including MRSA.^{420,422-426} There is an association between nasal MRSA colonisation and the carriage of MRSA on hands,^{56,427,428} with nasal colonisation thought to reflect longer term carriage.⁵⁶ There is a linear relationship between the length of time providing care and bacterial contamination of the hands of healthcare workers.^{425,429} Direct patient contact, respiratory care and contact with body fluid secretions in adults⁴²⁹ and skin contact, nappy change and respiratory care in neonates⁴³⁰ are associated with higher rates of bacterial hand contamination in healthcare workers. The use of gloves significantly reduces bacterial contamination of the hands,^{429,431} even when leaks occur.⁴³² Although there is a consensus on the benefit of cleaning hands of healthcare workers in reducing rates of nosocomial infection,^{425,433,434} findings on the relative superiority of available agents for hand cleansing differ. Some studies demonstrate improved outcome with hand washing if an antimicrobial agent such as chlorhexidine is used,⁴³⁵ and others find alcohol gel more effective.^{429,431} However, irritant contact dermatitis and eczema as a result of frequent and vigorous hand washing with anti-septic agents can result in broken skin and increased shedding of micro-organisms.⁴³⁴ Use of alcohol gel can increase hand cleansing compliance above that for hand washing with soap and water,⁴³⁶ likely due to its being easier to use and less time consuming. Intervention studies assessing the changes in nosocomial infections rates with hand hygiene programmes generally report a reduction in infection rates,⁴³⁷⁻⁴⁴¹ although not all reach significance.^{442,443}

Studies specifically addressing hand hygiene in resource-restricted South and East Asia are limited. Adequate hand washing is impeded in developing countries by a lack of basic resources such as soap and alcohol gel, as has been reported from Afghanistan and Cambodia;⁴⁴⁴ or lack of hand wash basins as detailed from Indonesia.^{445,446} Hand washing compliance remained poor even when soap and alcohol gel were readily available in a study from India.⁴⁴⁷ In Bangladesh, an intervention study that included hand hygiene amongst other infection control measures demonstrated a

reduction in the number of nosocomial infections in a special baby care unit.⁴⁴⁸ In Indonesia 2 different intervention studies both involving a combination of installing hand washing facilities and an education programme to promote hand washing increased hand washing compliance rates on the study wards.^{445,446} In the Philippines a study evaluating the interventions of providing alcohol hand gel, workshops on hand hygiene and a daily checklist of infection control aspects of care on 2 neonatal intensive care units found that whilst there was an improvement in hand washing compliance during the intervention period of the study compared with the previous observation period, the rates of colonisation with antibiotic resistant organisms, including MRSA, did not change significantly.⁴⁴⁹ A study reporting good hand hygiene compliance was published from Pakistan involving anaesthetists in a tertiary care hospital.⁴⁵⁰

1.3.2 Barrier nursing

Barrier nursing involves the use of gowns, gloves and masks for patient care and interaction with the patient's environment. These are recommended to prevent the patient's blood or bodily fluids coming into contact with the healthcare worker.^{406,451,452}

There are less data on barrier nursing than hand hygiene from the target countries. Two of the studies assessing hand washing compliance additionally investigated barrier nursing: one of the intervention studies from Indonesia also monitored the usage of gloves, gowns and masks and demonstrated that appropriate compliance was low, however overuse when not indicated was common;⁴⁴⁶ and the report published from Pakistan involving anaesthetists in a tertiary care hospital found good compliance with glove usage as well as hand hygiene.⁴⁵⁰

1.3.3 Isolation and cohorting

Infection control guidelines from developed countries recommend either isolating patients with MRSA or cohorting them to prevent further transmission of MRSA.^{406,452} Isolation refers to a single room whereas cohorting involves grouping patients with MRSA in a bay or geographically distinct area within a ward. In south-eastern Mediterranean hospitals

over-crowding and lack of isolation beds, especially in the context of high bed occupancy rates, have been demonstrated to result in higher prevalence rates of MRSA carriage.⁴⁵³ However, a study conducted in 2 Intensive Care Units in London did not find a benefit from cohorting or isolating patients with MRSA.⁴⁵⁴ There is a single paper published from South and East Asia relating to cohorting of patients. It involves an infection control intervention study performed in a special care nursery in Bangladesh which included cohorting neonates with surgical wound infections as well as carrying out other infection control measures, such as hand hygiene promotion, and found that infection rates were reduced following the interventions.⁴⁴⁸ The relative contribution of cohorting is not commented on.

1.3.4 Decolonisation

In certain circumstances, such as prior to major surgery involving prosthetic material, patients are decolonised of their MRSA in order to prevent subsequent infection.^{406,455} Decolonisation may also be used to help control an outbreak of MRSA.⁴⁰⁶ Typically decolonisation comprises nasal and skin decolonisation, which entails topical mupirocin applied to the anterior nasal passages 3 times a day for a 5 day course and washing with an antiseptic, such as chlorhexidine or povidine iodine, for 5 days.⁴⁰⁶ Mupirocin should only be used for short courses in order to prevent the development of resistance,⁴⁰⁶ hence when it is used in dialysis patients to prevent peritoneal catheter or intravenous catheter line infections intermittent courses are used rather than prolonged treatment.^{384,385} There is a single paper published from South and East Asia regarding the use of decolonisation to prevent infections in peritoneal dialysis patients in India.³⁷⁶ Gastrointestinal tract MRSA colonisation can represent a significant reservoir of MRSA since it can result in widespread environmental contamination.⁴⁵⁶ Gastrointestinal decolonisation with oral vancomycin has been successfully used to reduce MRSA transmission.⁴⁵⁷

1.3.5 “Search and destroy” policy

The prevalence of MRSA across Europe varies almost 100-fold, between the lowest rates seen in the Netherlands and Scandinavia (0.6-1%) and the highest rates in the UK (41.5%) and Greece (44.4%).⁴⁵⁸ These low rates of MRSA in the Netherlands and Scandinavia are attributed to their adoption of a “search and destroy” policy towards MRSA.^{459,460} This policy consists of actively screening for MRSA colonisation amongst patients and healthcare workers, isolation of colonised or infected patients to a single room with one allocated nurse, decolonisation of patients and healthcare workers until screening swabs are negative, healthcare workers are suspended from work until they are proven to no longer to carry MRSA, patients remain isolated until their screening swabs are negative, screening of the contacts of an index infected case and ward closure if more than 1 patient is infected or colonised on that ward.^{461,462} If a “search and destroy” policy is implemented in a hospital with endemic MRSA then it can take up to 6 years for the prevalence of MRSA to fall below 1%.⁴⁵⁹ A “search and destroy” policy is expensive to implement^{459,461,462} and therefore unlikely to be cost-effective for developing countries.

1.3.6 Antibiotic stewardship

Excessive and/ or inappropriate antibiotic use drives the emergence of drug-resistant strains.⁴⁰⁶ Work on the evolution of *S. aureus* clones has demonstrated that MSSA strains that are well adapted to transmission within hospitals repeatedly receive the *mec* determinant following the use of methicillin and related antibiotics,¹⁷⁰ which condones a survival advantage in the healthcare environment. Fluoroquinolones,^{463,464} cephalosporins^{465,466} and beta-lactamase penicillin antibiotics⁴⁶⁶ have all been associated with acquisition of MRSA. For this reason, antibiotic stewardship is an important component of MRSA infection control. This involves educating prescribers, instituting and auditing adherence to an antibiotic policy; preventing under-dosage of antibiotics and ensuring correct duration of antibiotic courses; consideration of antibiotic cycling; and reducing broad-spectrum antibiotics as much as possible, especially following culture results.^{406,467} Programmes focussing on antibiotic stewardship in developed countries

have significantly reduced rates of MRSA infection and colonisation.^{468,469} There is limited data on antibiotic stewardship from South and East Asia, although it is recognised as an important strategy.⁴⁷⁰ Antibiotic stewardship was one of a number of interventions involved in reducing nosocomial infection rates from a study in a special care nursery in Bangladesh.⁴⁴⁸ Antibiotic overuse has been recognised as a problem in China⁴⁷¹ and Nepal.^{67,472,473} In addition, sub-standard antibiotic drugs are rife across resource-restricted countries in South and East Asia¹⁹⁸ and these are likely to help drive drug resistance rates higher given the reduced amount of active drug present.

1.3.7 World Health Organisation challenge

The World Health Organisation launched the first of their Global Patient Safety Challenges in October 2005 with the slogan “Clean care is safer care”.⁴⁷⁴ The campaign focuses on reducing healthcare-associated infections. The aim of the challenge is to promote safe hand hygiene practices globally and at all levels of healthcare as a first step in ensuring high standards of infection control and patient safety. It included producing guidelines on hand hygiene for use globally. The resource-restricted countries from South and East Asia which have signed up to the challenge are Bangladesh, Maldives, Mongolia, Thailand and Vietnam.

1.4 Aims of this thesis

Overall, the work described in this thesis was aspiring to raise the profile of *S. aureus* disease as an important pathogen in developing countries, using Thailand as an example of a resource-restricted country from South and East Asia. More specifically my aims were to:

1. Define the epidemiology of *S. aureus* disease in the tropics.
2. Determine the clinical manifestations of *S. aureus* disease in the tropics.
3. Ascertain the rate of MRSA infection, the antibiotic sensitivity profiles and presence of community-acquired MRSA in a resource-restricted country.
4. Elucidate the impact of MRSA on antibiotic prescribing and its efficacy.
5. Define the *S. aureus*-attributable mortality rate and the factors that predict mortality in a resource-restricted country.
6. Establish whether implementation of the "Surviving Sepsis Campaign" guidelines would be feasible in a resource-restricted setting.
7. Determine the prevalence of Panton-Valentine Leukocidin amongst *S. aureus* isolates causing disease in an unselected population.
8. Define rates of MRSA carriage amongst patients and nursing staff of Intensive Care Units and likely modes of transmission.

Chapter 2. Methods and materials

2.1 Retrospective clinical study

2.1.1 Participants and clinical methods

Ethical approval was obtained from the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand and the Oxford Tropical Research Ethics Committee. A retrospective, observational study of *S. aureus* bacteraemia was performed at Sappasithiprasong Hospital in northeast Thailand, which lies 70 km west of Lao PDR and 95 km north of Cambodia. This hospital serves the province of Ubon Ratchathani with a catchment of 2 million people, providing both primary care to the town inhabitants and tertiary care to the inhabitants of the province. It is a 1,100-bed hospital with comprehensive clinical and laboratory services.

The computerised records of the hospital diagnostic microbiology laboratory were interrogated to identify all patients with *S. aureus* grown from a blood culture between 1st June 2003 and 31st December 2004. In-patients of all ages with a pure growth of *S. aureus* were eligible for inclusion. Clinical paper notes were available from the hospital medical records department on 135 out of 157 patients (86%), with limited data available from hospital computer records for the remaining 22 cases. The paper notes were used to collect information on patient characteristics, past medical history, presenting symptoms and examination, cultures taken and results, investigation results, sites of infection, antibiotic treatment and other management, and outcome. The data available on the computerised records were culture details including ward of collection, admission and discharge dates, date of birth, sex, dates of subsequent hospital attendance and death. Additional out-of-hospital deaths were established from checking the National Death Register. Data were recorded on standardised forms adapted from those used by Fowler et al¹⁶¹ (Appendix 1). Final outcome was determined 12 weeks from the date of the first positive blood culture result for *S. aureus*, in keeping with the Fowler et al study.¹⁶¹

2.1.2 Definitions

Community-acquired infection was defined as a positive *S. aureus* blood culture taken within 2 days of admission to hospital. Nosocomial infection was defined as a positive *S. aureus* blood culture taken more than 2 days after admission.

2.2 Prospective clinical study

2.2.1 Participants and clinical methods

Ethical approval was obtained from the Ethical and Scientific Review sub-committee of the Royal Thai Government Ministry of Public Health and the Oxford Tropical Research Ethics Committee. A prospective, observational study of invasive *S. aureus* disease was conducted at Sappasithiprasong Hospital in northeast Thailand over a 1 year period from November 2006 to November 2007. Potential study patients were identified by daily consultation with the hospital diagnostic microbiology laboratory. Patients of all ages with at least one clinically significant culture sample taken from a normally sterile site positive for a pure growth of *S. aureus* were considered for inclusion. Blood culture was considered to represent a sterile site sample, and other sample types were determined based on details in the clinical notes and discussion with hospital staff. Surface swabs and other samples that were not collected from usually sterile sites or not obtained via aspiration or an operative procedure were excluded. Patients were enrolled into the study after written informed consent was obtained, and were visited daily by study investigators until discharge to record progress and management.

More detailed information could be collected compared with the retrospective study since the patient and/or relatives were interviewed, the patient examined by the study investigators, and hospital staff (especially the ward nurses) were able to elaborate on management plans which were usually scantily documented. Data were recorded on standardised forms adapted from those used by Fowler et al¹⁶¹ and further modified from the retrospective study to accommodate the greater detail (Appendix 2). Additional data was collected on the severity of illness and sepsis management for the 3 day period

starting from the day the first culture positive for *S. aureus* was taken, a time frame chosen to reflect the 72 hours of early goal-directed therapy for sepsis described by Rivers et al³¹² (Appendix 3). The aspects of sepsis management evaluated were those detailed in the 'Surviving Sepsis Campaign' guidelines published in 2004.³³⁹ However, these guidelines were revised and published before the analysis of these data and hence were assessed against the 2008 updated guidelines.³⁴⁰ Clinical care was provided by the hospital physicians, independent of the research team. Sites of *S. aureus* infection were established based on examination findings and referral to the medical notes, including investigation reports and operation notes. Final outcome was determined 12 weeks from the date the first culture positive for *S. aureus* was taken, in keeping with the Fowler et al study,¹⁶¹ using a standardised telephone questionnaire.

The study-specific investigations performed were a repeat blood culture taken 48-96 hours after the first culture positive for *S. aureus* was taken and a transthoracic echocardiogram. Results of these study investigations were given to hospital physicians caring for the patient. Echocardiograms on patients aged less than 14 years were performed by the hospital paediatric echocardiographer, while echocardiograms on the remainder were performed by a study investigator and reviewed or repeated as appropriate by the hospital cardiologist. Subsequently the echocardiograms carried out by the study investigator were reviewed by a cardiologist in Oxford.

2.2.2 Definitions

Community-acquired infection was defined as a positive *S. aureus* sterile site culture and admission to hospital with an illness consistent with invasive *S. aureus* disease.

Nosocomial infection was defined as a positive *S. aureus* sterile site culture taken more than 48 hours after admission for another condition. Non-nosocomial healthcare-associated infection was defined as community-acquired infection in an individual who had contact with healthcare services in the preceding year, using the criteria described by Fowler et al.¹⁶¹ Outcomes at 12 weeks were defined as: (i) cure - clinically improved and no additional sites of infection present or suspected; (ii) unresolved infection - persistent

features of infection with or without persistent positive cultures; (iii) death attributable to *S. aureus* - when death was due to *S. aureus* infection in a previously healthy individual or when *S. aureus* hastened death in the presence of an underlying condition such as cancer; or (iv) death due to other causes - in which the *S. aureus* infection did not appear to contribute to death.

2.3 Laboratory methods for the clinical disease studies

2.3.1 Hospital diagnostic microbiology laboratory

The hospital diagnostic microbiology laboratory processed all samples using routine procedures, including antibiotic susceptibility testing using the disk diffusion method.⁴⁷⁵ Susceptibilities reported to hospital physicians were penicillin, oxacillin, cefazolin, erythromycin, clindamycin and trimethoprim-sulphamethoxazole for methicillin-susceptible *S. aureus* (MSSA). Methicillin-resistant *S. aureus* (MRSA) were additionally tested for vancomycin and fusidic acid. For the retrospective study, isolates were determined as MRSA based on the oxacillin disk diffusion assay.

2.3.2 Antibiotic susceptibility testing in research laboratory

In the prospective study, a total of 248 out of 270 *S. aureus* isolates (first positive sample for each patient, with preference given to blood culture samples when two different samples taken on the same day were both positive) were available and obtained for further testing, re-identified and stored at -80°C. Species verification was ascertained using coagulase and latex agglutination (Staphaurex Plus) tests. Isolates were evaluated against an extended antibiotic panel of ceftiofur, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, mupirocin, netilmicin, penicillin, rifampicin, trimethoprim-sulphamethoxazole, tetracycline and vancomycin using the disk diffusion method.⁴⁷⁵ Isolates that were resistant to ceftiofur by disk diffusion were further evaluated by oxacillin and vancomycin E-tests (AB Biodisk). Isolates from the prospective study were designated as MRSA based on the oxacillin E-test for the 248 isolates re-tested in the research laboratory, and based on the oxacillin disk diffusion assay for the 22

isolates that were tested by the hospital laboratory and were not available for further testing.

2.3.3 Polymerase chain reaction (PCR) in research laboratory

A multiplex polymerase chain reaction (PCR) was used to determine the presence of *mecA*, the genes encoding Panton-Valentine Leukocidin (PVL) and the 16S rRNA gene (internal positive control). A 1ml overnight culture of *S. aureus* with an optical density of 1.0 at 600nm was centrifuged at 16,000×g for 30 seconds and the cell pellet re-suspended with 200µL phosphate buffer solution (pH 7). Genomic DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Applied Science, Germany), with an additional step of incubation at 37°C for 30 minutes with 0.5µL of 10mg/ml lysostaphin solution (Sigma, USA) prior to cell lysis. The primers used were as described previously for mA1 and mA2 (*mecA*),⁴⁷⁶ luk-PV-1 and luk-PV-2 (PVL),²⁶⁶ and Staph756F and Staph750R (*Staphylococcus* genus-specific 16S rRNA gene).⁴⁷⁷ A 25µL PCR reaction was used containing 10-120ng genomic DNA, 5pmol of each primer (Proligo Singapore Pty Ltd), 200µM dNTP (Qiagen, Germany), 1.25 units of Taq polymerase (Roche Applied Science, Germany), 4.5mM MgCl₂ and 1×buffer. PCR was performed using a PTC-200 Peltier Thermal Cycler (MJ research, USA). The PCR conditions were one cycle of 95°C for two minutes, 30 cycles of 15 seconds at 95°C, 15 seconds at 56°C; 45 seconds at 72°C, and a final step of 72°C for seven minutes. Amplification product size was determined by reference to a 100-bp molecular weight marker (Biolabs New England, UK) using 2% agarose gel electrophoresis.

2.4 MRSA carriage study

2.4.1 Participants and clinical methods

Ethical approval was obtained from the Ethical and Scientific Review sub-committee of the Royal Thai Government Ministry of Public Health and the Oxford Tropical Research Ethics Committee. A prospective, observational study of MRSA carriage and transmission was conducted in 2 Intensive Care Unit (ICU) wards at Sappasithprasong Hospital in

northeast Thailand for a period of 3 months, from 1st March 2008 to 31st May 2008. The study ICU wards were a general paediatric ICU, which contained 7 beds and 7 cots in open plan, and an adult surgical ICU that had 8 beds in open plan. These wards were chosen as they had harboured patients with MRSA bacteraemia in the previous prospective clinical study. All patients admitted to the study ICU wards were eligible for inclusion and patients were enrolled into the study after providing written informed consent. Data was recorded for each patient with particular emphasis on ward locations prior to the study ICU, antibiotic treatment and development of any nosocomial infection (Appendix 4).

Screening swabs of the anterior nares, throat, axillae and wounds if present, were taken using pre-moistened sterile cotton-tipped swabs on admission (or within 24 hours of admission), then twice weekly during the length of their stay on the study ward and on discharge from this ward. At the same time points those patients who were catheterised had a 1ml sample of urine aspirated from their catheter tubing and those patients who were intubated had a tracheal suction sample taken instead of a throat swab.

The nursing staff working on the study ICU wards were eligible for inclusion and were enrolled into the study after providing written informed consent. Basic demographic information, including their nursing grade and number of years working, was recorded. Nursing staff had screening nasal swabs and finger tip cultures at 3 unannounced time points over the study period. The finger tip cultures were obtained by the direct placement of the finger tips of the nurse's dominant hand onto a Columbia blood agar plate.⁴²⁹ Covert surveys of hand washing were performed every 2 weeks throughout the study on each study ICU ward using the Hand Hygiene Observation Tool.⁴⁷⁸

2.4.2 Laboratory methods

After swabbing the end of the swab was broken off into a bijou with 1ml of sterile phosphate buffer solution (PBS). After returning to the laboratory (and within two hours of sampling), the bijou were vortexed and then 100µL (10µL only for nasal swabs) of swab fluid was spread inoculated onto a phenol red mannitol salt agar plate and the remainder

of the fluid, including the swab tip was added to 7ml phenol red mannitol salt agar broth. The agar plates were incubated for 48 hours and the broth for 7 days at 37°C in air. If no colonies were identified on the agar plate then the broth was checked for a change in colour from red to yellow at 48 hours and 7 days, which if observed then 10µL broth was plated to phenol red mannitol salt agar. Suspected colonies on agar were verified using coagulase and latex agglutination tests. The finger imprint Columbia blood agar plates were incubated for 48 hours at 37°C in air and suspected colonies processed as with the swab plates. Methicillin susceptibility was tested using a ceftiofur disk on Mueller-Hinton agar.

On return to the laboratory (within 2 hours of sampling), the section of tubing from tracheal suction sampling in its universal container was vortexed to obtain the sputum, after which the tubing was discarded. 1ml sterile PBS was added to the sputum and vortexed then the sample was processed in the same manner as the swab samples. The 1ml urine samples collected by syringe from the catheter tubing were placed into a bijou and on return to the laboratory 10µL was spread inoculated neat onto the phenol red mannitol salt agar plate and cultured for 48 hours at 37°C in air. The remaining urine was spun to obtain a pellet and the pellet added to the phenol red mannitol salt agar broth, which was incubated for 7 days at 37°C in air. Then the urine samples were processed in the same manner as the swab samples.

Isolates were stored at -80°C and subsequently an extended antibiotic susceptibility profile was determined using the disk diffusion method⁴⁷⁵ for chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, mupirocin, netilmicin, penicillin, rifampicin, trimethoprim-sulphamethoxazole, tetracycline and vancomycin. Isolates that were resistant to ceftiofur by disk diffusion were evaluated by oxacillin E-tests (AB Biodisk). The first MRSA isolate for each patient additionally underwent multiplex PCR for *mecA*, 16S ribosomal RNA (internal control) and the gene encoding PVL, as described above in section 2.3.3.

2.4.3 Results confidentiality

The results of cultures grown from the swabs of patients as part of the study were kept confidential since the swabbing of patients to look for MRSA colonisation is not routine practice on either of the study wards and isolation of infected or colonised patients is not possible. Additionally, all the results of cultures grown from nursing staff were kept confidential.

2.5 Statistical analysis

2.5.1 Retrospective clinical study

Data was entered into Excel spreadsheets. Data analyses were performed using Stata software version 9 (StataCorp, College Station, Texas). Fisher's exact test was employed to compare categorical data. Continuous data was compared with Student's t test because the standard deviations were similar, even though the data was not normally distributed.

2.5.2 Prospective clinical study

Data was double entered into a FileMaker Pro database (version 7). Data analyses were performed using Stata software version 9 (StataCorp, College Station, Texas). Categorical data were compared using Fisher's exact test. Student's t test was used for continuous data when the data was normally distributed or the standard deviations were similar; otherwise the Mann-Whitney U test was employed. Kaplan-Meier survival curves were plotted for attributable and non-attributable deaths, and for *S. aureus*-attributable deaths in children and adults (non-attributable deaths censored). These survival curves were compared using the log rank test. In order to determine the risk factors for mortality, variables significant at $p < 0.20$ on univariable analysis and with 5 or more events (deaths) were used in the multivariable logistic regression model. Superficial and deep abscesses were combined so that there were 5 events. Prematurity was excluded given the low number of cases overall ($n=7$). Variables which did not reach this threshold were reviewed

and those considered a priori to potentially have an important influence on outcome were added (diabetes mellitus was the only variable in this category). Stepwise removal and addition of variables were used to determine the final multivariable model (p for removal 0.1 and p for re-entry 0.05). Missing PVL data was excluded from the multivariable logistic regression. Potential interactions were assessed using logistic regression. Reported p values are two-tailed.

2.5.3 MRSA carriage study

Data was entered into Access databases. Data analyses were performed using Stata software version 9 (StataCorp, College Station, Texas). Categorical data were compared using Fisher's exact test. Student's t test was used for continuous data with a normal distribution; otherwise the Mann-Whitney U test was employed.

Chapter 3. Retrospective study of *S. aureus* bacteraemia in a tropical setting

3.1 Chapter content

Invasive *S. aureus* disease is poorly understood in tropical settings. A study specifically evaluating *S. aureus* bacteraemia, rather than all-cause bacteraemia, had not been reported in the English language literature from resource-restricted South or East Asia prior to the work for this thesis. This retrospective observational study of *S. aureus* bacteraemia was performed to assess the burden of morbidity and mortality in a resource-restricted tropical setting. In addition, the data generated formed the basis for the development of hypotheses and informed the design of a subsequent prospective study of invasive *S. aureus* disease described in Chapters 4-6. Study methods were described in Chapter 2.

3.2 Results

The quality of the documentation in the hospital patient notes was low. The handwriting was sometimes illegible and some patients had no record of history and/or examination findings. Amongst the scanty progress notes, there was no documentation provided to explain antibiotic choice or change of treatment in any of the cases. For 135 of the 157 cases identified, there were hospital paper patient records available and for the remaining 22 cases basic data was obtained from the hospital electronic records.

3.2.1 Patient characteristics

Over the 19 month period considered for the study, there were 157 cases of *S. aureus* bacteraemia identified from the hospital diagnostic microbiology laboratory electronic database. The age range of patients on the day their culture was taken was from 1 day of life to 94 years (median 33 years, interquartile (IQR) range 1 to 59 years). The largest age group was patients aged less than 1 year (25%); the age distribution of the study patients is shown in decade blocks in Figure 3.1. Of the 39 patients aged less than 1 year, 27 (69%) were neonates (aged less than 1 month) at the time of their culture. There was a

significant predominance of males (n=98, 62%, p=0.002). Amongst the 135 patients with paper records, a chronic underlying illness was detailed in the notes of 47 patients (35%) although 17 patients (13%) had no documentation relating to past medical history. The most common underlying diseases recorded were diabetes mellitus (n=19) and chronic renal failure (n=15). The median length of stay for all patients was 9 days (IQR 5 to 19 days), with 32 patients (20%) admitted for 3 days or less. The cases of *S. aureus* bacteraemia occurred throughout the hospital.

3.2.2 Types of infection

For nearly half the patients with paper records, it was not possible to identify a site of infection beyond bacteraemia (n=66; 49%). However, amongst the cases where it was possible a broad spectrum of sites of infection was seen including invasive disease such as pneumonia, septic arthritis, pyomyositis and endocarditis as well as more superficial infections like those affecting the skin and soft tissue (Table 3.1). A total of 72 sites of infection were found from 69 patients; 3 patients each had 2 identified sites. The most common sites were skin and soft tissue infection (21 sites, 29% of all sites) and pneumonia (17 cases, 24%). Although there were 13 patients with infection related to prosthetic material and/ or surgery, in a further 9 cases there was prosthetic material present or there had been a preceding operation but the documentation was not sufficient to determine whether the bacteraemia arose as a result of these.

Table 3.1: Sites of infection

Site of infection	Number of sites (n=72) (% of total sites)
Skin and soft tissue infection	21 (29%)
Pneumonia ^{*1}	17 (24%)
Infection related to prosthetic material and/ or surgery ^{*2}	13 (18%)
Septic arthritis	7 (10%)
Pyomyositis	5 (7%)
Urinary tract infection	4 (6%)
Other infections ^{*3}	3 (4%)
Endocarditis	2 (3%)

^{*1} 2 cases of pneumonia also had empyema

^{*2} Infection related to prosthetic material and/ or surgery includes: central intravenous catheter (n=4), haemodialysis graft (n=2), umbilical vein catheter (n=1), pacemaker (n=1), post metallic mitral valve replacement (n=1), following closure of 7mm atrial septal defect (ASD) (n=1), after pericardiectomy and insertion of mediastinal drain (n=1), post above knee amputation (n=1) and following orthopaedic operation and insertion of metalwork (n=1).

^{*3} Other infections category includes: liver abscess (n=1), central nervous system infection (n=1) and sepsis following an out-of-hospital abortion (n=1).

The number of investigations recorded in the paper notes was low. A transthoracic echocardiogram was performed on 13 patients, although the reports from 8 of these did not mention vegetations or endocarditis. There were 2 patients with metallic heart valves and *S. aureus* bacteraemia, neither of whom had a transthoracic echocardiogram performed. A documented search for possible collections of pus or other sites of infection was recorded in the notes of a single patient. From the 135 patients with paper records available, 44 patients (33%) had a repeat blood culture taken (after the day the first culture positive for *S. aureus* was taken), from which 7 (16%) were positive for *S. aureus*.

3.2.3 Drug resistance

The rate of MRSA infection was 23% of all cases (n=36). There were 48 cases of hospital-acquired infection, of which 28 (58%) were caused by MRSA. There were 8 “community-acquired” cases of MRSA, but all of these patients had attended Sappasithiprasong Hospital in the preceding year, with 7 having been in-patients.

3.2.4 Antibiotic treatment

The median duration of in-patient antibiotic treatment was 8 days (IQR 4-17 days) amongst the 135 patients with paper notes. The choice of antibiotics prescribed was highly variable and poly-pharmacy was commonplace. Considering cloxacillin and vancomycin as the gold standard treatment for MSSA and MRSA bacteraemia respectively, 53 patients (44%; 19 (16%) unknown treatment) infected with MSSA and 16 patients (44%; 3 (8%) unknown treatment) infected with MRSA received the gold standard choice of antibiotic for their bacteraemia. Amongst the 16 patients treated with vancomycin, 3 received their first dose of vancomycin on the day the positive culture was taken, whilst the median delay was 5 days (IQR 3-6 days) for the remaining 13 patients. Given the wide variety of antibiotics prescribed and the limited antibiotic susceptibility profile reported by the hospital diagnostic microbiology laboratory, it was not possible to accurately determine the proportion of patients who received effective antibiotic therapy for their *S. aureus* bacteraemia and time delays to effective treatment for MSSA patients.

3.2.5 Procedures for infectious source control

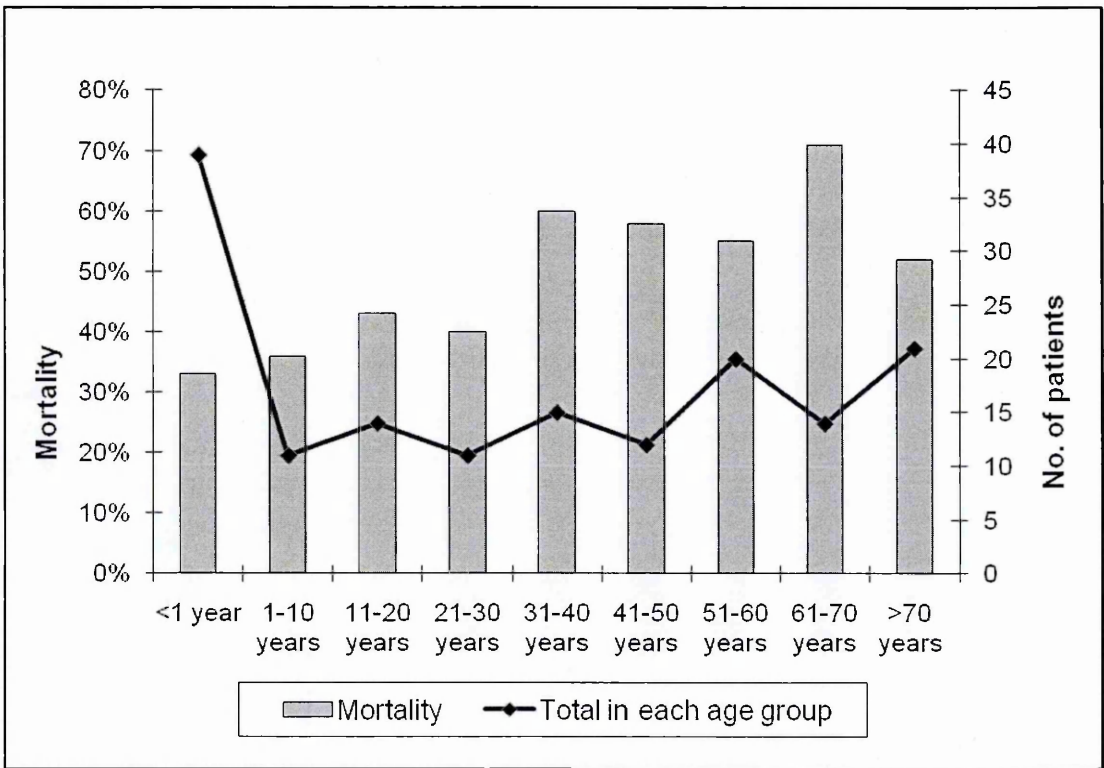
Procedures for infectious source control were recorded on 8 patients (6% of those with paper records). The procedures were: joint washout (n=3), debridement (n=3), incision and drainage (n=1) and revision of pacemaker (n=1).

3.2.6 Mortality

The overall mortality rate at 12 weeks was 48%. Age-specific mortality is shown in Figure 3.1. There was a highly significant association between increasing age and mortality ($p=0.001$). Amongst those patients with paper medical records and documented details on the presence or absence of chronic underlying diseases (n=118), there was a significant association between the documented presence of chronic underlying diseases and death; mortality rates were 60% and 39% for presence and absence, respectively ($p=0.039$). The mortality rate for infection with MRSA was significantly higher than for MSSA, 67% versus 43%, respectively ($p=0.014$). Amongst the patients with paper medical records (n=135), there was a trend towards a reduced mortality if a site of infection in addition to bacteraemia was identified; mortality was 42% in those with an identified site of infection compared with 58% in those without ($p=0.086$).

Figure 3.1: Age distribution and age-specific mortality rates and disease burden for 157 cases of *S. aureus* bacteraemia.

The burden of *S. aureus* bacteraemia is highest amongst the youngest age group (<1 year) whilst overall mortality increased significantly with age ($p=0.001$), analysing age as a continuous variable.



3.3 Discussion

This retrospective study has demonstrated that *S. aureus* bacteraemia is an important cause of morbidity and mortality in the resource-restricted tropical setting of the study hospital. Over the study period *S. aureus* accounted for 9% of positive blood cultures, the third most common cause after *Escherichia coli* (17%), and *Burkholderia pseudomallei* (the cause of melioidosis) (14%). The overall mortality was double that found in studies from the USA (28% mortality in one large study;¹⁶¹ 30% in those aged over 65, and 15% overall in adults aged 18-60 years in a second study).⁴⁷⁹ The mortality rate is similar to death rates following bacteraemic melioidosis, which is the other major cause of bacteraemia and sepsis-related death in Ubon Ratchathani.^{480,481} Follow-up data after discharge from hospital was limited as a result of the retrospective study design and was only available if the patient returned to Sappasithiprasong Hospital either as an in-patient or an out-patient. National death registry information was used to more accurately verify the mortality rate. Therefore the data available does not support the ascertainment of an accurate attributable mortality rate.

Mortality increased significantly with age and the presence of chronic underlying diseases, which would also be expected to increase with age. Given the limited data on chronic underlying diseases, reducing the number of cases in logistic regression from 157 to 115, the effect of potential confounding between age and chronic underlying diseases could not be accurately determined. More complete data on chronic underlying diseases would facilitate the investigation of the significance of this potential confounding effect.

MRSA was a significant problem in the study hospital, and the death rate of patients infected with MRSA was extremely high, significantly more than for MSSA. Less than half the patients received gold standard antibiotic therapy for their bacteraemia but given the wide variety of antibiotics used and the limited antibiotic susceptibility profiles available it was not possible to accurately establish the proportion of patients receiving effective antibiotic therapy, either empirically or following culture results. Delays in effective antibiotic therapy for *S. aureus* bacteraemia are known to adversely affect outcome¹⁸⁶ and the delays in receiving vancomycin for the majority of patients infected

with MRSA may have contributed to the higher mortality rate for MRSA. Although the median duration of antibiotic treatment appears shorter than that usually recommended for bacteraemia, because of the retrospective design it was only possible to record the antibiotic course received in the study hospital. Therefore meaningful conclusions over antibiotic treatment duration and its relationship to outcome were not possible. It was only practical to base the differentiation of community-acquired infection versus hospital-acquired infection on the timing of cultures compared with admission. This does not take account of possible healthcare-associated infection arising whilst the patient is not admitted to hospital and can be an important source of MRSA.

The broad range of sites of infection recorded is in contrast to the textbook¹⁵⁶⁻¹⁵⁸ focus on pyomyositis as the 'tropical' form of *S. aureus* disease. Of note in this study there were just 5 cases of pyomyositis seen. However, the comparatively low rate of identified sites of infection beyond bacteraemia is not consistent with the very high death rate and indicates that these may be under-recognised and under-investigated. For example, there were no cases of osteomyelitis, and only one solid organ abscess and 2 cases of endocarditis. From this retrospective study it was not feasible to distinguish whether investigations were carried out and not recorded, or were not performed. On a similar note, there was a very low rate of procedures for infectious source control.

There are inherent limitations of a retrospective study and these were further exacerbated by the lack of and poor quality documentation of hospital paper records and no access to the infecting isolates. Nevertheless, the study provided useful data to inform the design of a future prospective study. It was clearly going to be essential to be able to interview the patients and their relatives to obtain fuller details on the presentation of the infection, underlying chronic medical conditions and previous healthcare contact, as well as being able to examine the patient. The ability to contact the patient by phone or letter to accurately establish follow-up data, further antibiotic treatment and outcome would be invaluable. Discussions with hospital staff about management in addition to referral to the medical notes, and having radiological images available on the ward, would also be expected to enhance the quality of the data considerably. In addition, in a prospective

study it would be achievable to include all culture samples from usually sterile sites because the source of the sample could be ascertained from the hospital staff or patient if the notes were not sufficiently detailed. This would be predicted to give a more accurate picture of the manifestations of invasive *S. aureus* disease in a tropical setting, rather than just focussing on bacteraemic patients. Furthermore, the infecting isolates would be saved and evaluated further in the research laboratory in order to determine a more detailed antibiotic susceptibility profile, and molecular techniques undertaken to define the presence of the *mecA* gene for MRSA confirmation and the presence of the putative virulence determinant Panton-Valentine Leukocidin. Information would also be collected on occupation, as it could be a possible explanation for the significantly higher number of males seen in this study.

Hypotheses developed as to possible causes for the high mortality rate found were lack of investigations to identify sites of infection and pus collections, lack of procedures for infectious source control and inappropriate antibiotic prescribing. Specific investigations to be introduced in the prospective study were a transthoracic echocardiogram for every patient with bacteraemia since endocarditis can complicate *S. aureus* bacteraemia and has a high mortality rate,⁴⁸² and a repeat blood culture taken 48-96 hours after the first culture positive for *S. aureus* since this has been shown to be the most effective predictor of complications following *S. aureus* bacteraemia in a US based study carried out by Vance Fowler et al.¹⁶¹

Chapter 4: Prospective study of risk factors for death and interventions to reduce mortality

4.1 Chapter content

The retrospective study of *S. aureus* bacteraemia, described in Chapter 3, established that *S. aureus* was responsible for considerable morbidity and mortality in a resource-restricted tropical setting. However, there were limitations to the data due to the retrospective nature of the study. The design of this prospective observational study of invasive *S. aureus* disease was informed by the retrospective study and devised to undertake a more detailed assessment of the risk factors for mortality and interventions to reduce mortality. This prospective study was carried out in the same study hospital in northeast Thailand. Study methods were described in Chapter 2.

4.2 Results

4.2.1 Recruitment

Over the 1 year study period a total of 295 patients were identified with at least one clinically significant culture from a normally sterile site positive for a pure growth of *S. aureus*. Of these, 270 patients were recruited and 25 patients could not be studied either because they declined consent (n=21) or because they had left the hospital by the time the culture became positive and could not be contacted (n=4).

4.2.2 Culture samples

From the 270 study patients there were 335 sterile site cultures positive for *S. aureus*, of which 201 were pus specimens obtained by aspiration or from operative procedures, 125 were blood cultures, 5 were pleural fluid, 3 were cerebrospinal fluid (CSF), and one was peritoneal dialysate. Considering patients as the denominator, 100 patients (37%) had positive blood cultures of whom 14 had at least one other positive sterile site culture from an identified focus of infection, and 170 patients had positive pus culture(s) alone.

4.2.3 Outcome

The all-cause mortality rate at 12 weeks was 26% (70/270), of which 20% (55/270) were attributable to *S. aureus* infection. Overall mortality for bacteraemic patients was 53%, compared with 10% in patients without a positive blood culture ($p<0.001$). Mortality rates were 16% (14/87) for children and 31% (56/183) for adults ($p=0.01$), of which 14% and 25% were attributable to *S. aureus*, respectively. The majority of infections (191, 71%) were community-acquired, of whom 34 (18%) died (Table 4.1). The remainder comprised 27 (10%) cases of non-nosocomial healthcare-associated infection of whom 9 (33%) died, and 52 (19%) cases of nosocomial infection of whom 27 (52%) died ($p<0.001$ for comparison of 3 groups). Most of the 200 patients who survived to follow up at 12 weeks were cured ($n=189$). The remaining 11 patients had a range of unresolved *S. aureus* infections: empyema ($n=2$), diabetic foot infection ($n=2$), infected orthopaedic material ($n=2$), infected pacemaker with endocarditis ($n=1$), osteomyelitis plus pyomyositis with ($n=1$) or without ($n=1$) septic arthritis, septic arthritis alone ($n=1$) or orbital abscess ($n=1$).

4.2.4 Patient characteristics

The patient characteristics are summarised in Table 4.1. Age on the day the first culture positive for *S. aureus* was taken ranged from 1 day to 92 years (median 39 years). Overall and attributable mortality were significantly associated with increasing age ($p<0.001$ for both), and overall mortality but not attributable mortality was associated with prematurity ($p=0.014$). The majority of patients (98%) were Thai and the remaining 5 patients were from neighbouring Lao PDR. Three quarters of patients over the age of 16 years were manual labourers (a reflection of the major form of employment for people living in this region), but this group were not at increased risk of death. Almost half of patients (49%) had one or more of the underlying medical conditions that were recorded during the study, of which diabetes mellitus was the most frequent. The presence of pre-existing cardiac, renal or lung disease was significantly associated with all-cause mortality and pre-existing cardiac and renal disease were significantly associated with attributable mortality. However no association was found between death and either diabetes mellitus or

immunosuppression. The positive association between increasing age and mortality remained significant when adjusted for all underlying co-morbidities ($p=0.01$), pre-existing cardiac ($p=0.001$), renal ($p=0.001$) or lung disease ($p<0.001$).

4.2.5 Clinical manifestations

The majority of the 270 patients ($n=231$, 86%) had one or more identifiable extra-vascular sites of infection, while no site of infection was identified other than positive blood culture in the remaining 39 cases (Table 4.2). Superficial and deep abscesses accounted for the majority of infections (34% and 17%, respectively). A wide range of other clinical presentations were represented, including bone and joint infection, respiratory infection, endocarditis and nosocomial infections including post-operative wound infection and infections related to the presence of prosthetic material. Superficial and deep abscesses were strongly associated with a survival benefit ($p<0.001$ and $p=0.004$, respectively), while respiratory infections and having a blood culture positive for *S. aureus* were strongly associated with all-cause and attributable death ($p<0.001$ for all comparisons). Patients with an identified extra-vascular site of infection were less likely to die than those patients with bacteraemia alone (all-cause 20% vs. 59%, $p<0.001$; attributable 16% vs. 56%, $p<0.001$).

Table 4.1: Association between patient characteristics and outcome for 270 patients with invasive *S. aureus* infection

	Overall (n=270)	Survivors (n=200)	All-cause deaths (n=70)	All-cause p value* ¹	S. aureus-attributable deaths (n=55)	Attributable p value* ²
	Number, % of overall patients	Number, % of survivors	Number, % of all deaths		Number, % of attributable deaths	
Demographics						
Age (years): median (IQR)	39 (14-60)	35 (13-53)	55 (32-70)	<0.001	56 (30-68)	<0.001
Sex (male)	163 (60%)	121 (61%)	42 (60%)	>0.999	34 (62%)	0.878
Manual labour* ³ (if aged over 16 years)	147 (77%) (n=191)	101 (75%) (n=134)	46 (81%) (n=57)	0.459	34 (77%) (n=44)	>0.999
- Rice farming* ³	102 (53%) (n=191)	72 (54%) (n=134)	30 (53%) (n=57)	>0.999	21 (48%) (n=44)	0.396
Co-morbidities						
Prematurity/ Very low birth weight	7 (3%)	2 (1%)	5 (7%)	0.014	3 (5%)	0.152
Underlying medical conditions* ⁴	131 (49%)	81 (41%)	50 (71%)	<0.001	39 (71%)	<0.001
- Diabetes mellitus	42 (16%)	31 (16%)	11 (16%)	>0.999	10 (18%)	0.536
- Immunosuppression* ⁵	30 (11%)	20 (10%)	10 (14%)	0.377	8 (15%)	0.345
- Renal disease* ⁶	25 (9%)	12 (6%)	13 (19%)	0.003	10 (18%)	0.017
- Cardiac disease* ⁷	24 (9%)	5 (3%)	19 (27%)	<0.001	15 (27%)	<0.001
- Lung disease* ⁸	16 (6%)	8 (4%)	8 (11%)	0.036	4 (7%)	0.748
Source of infection						
Community-acquired	191 (71%)	157 (79%)	34 (49%)		25 (45%)	
Non-nosocomial healthcare-associated	27 (10%)	18 (9%)	9 (13%)	<0.001	7 (13%)	<0.001
Nosocomial	52 (19%)	25 (13%)	27 (39%)		23 (42%)	
Drug resistance						
MRSA	42 (16%)	19 (10%)	23 (33%)	<0.001	17 (31%)	0.001
Interventions						
Effective antibiotic therapy without delay	220 (81%)	175 (88%)	45 (64%)	<0.001	34 (62%)	<0.001
Procedure for infectious source control	184 (68%)	162 (81%)	22 (31%)	<0.001	13 (24%)	<0.001

Data are number (%) unless otherwise stated.

*¹ p value for the comparison between all-cause deaths and survivors.

*² p value for the comparison between *S. aureus*-attributable deaths and all other patients (survivors and other deaths combined).

*³ Denominator for occupation is number of patients over the age of 16 years which is given in each square.

*⁴ Past medical history of any underlying chronic medical conditions reported by the patient/relative or recorded in the medical notes.

*⁵ Immunosuppression from HIV (5 untreated, 3 on anti-retroviral therapy), chemotherapy (n=3), untreated leukaemia (n=1), radiotherapy (n=1) or immunosuppressive medication including prednisolone more than 30mg/day for more than 1 week (n=17).

*⁶ Renal disease included end stage renal failure on long-term dialysis (n=3; 2 on haemodialysis, 1 on peritoneal dialysis) and chronic renal failure (not on dialysis) due to diabetes mellitus (n=14), systemic lupus erythematosus (n=1), multiple myeloma (n=1), glomerulonephritis (n=1) or an unknown aetiology (n=5).

*⁷ Cardiac disease comprised congenital heart disease (n=4), valvular heart disease including rheumatic heart disease (n=8), ischaemic heart disease (n=8), or arrhythmias including heart block requiring pacemaker (n=4).

*⁸ Lung disease comprised previously treated tuberculosis (n=9), previous empyema (n=1), lung cancer (n=2), long-term tracheostomy (n=1), chronic obstructive pulmonary disease (n=2) or asthma (n=1).

Table 4.2: The range of sites of infection in patients and outcome associated with each clinical presentation

Clinical presentations	Total no. of sites (n=264)		Total no. of patients (n=270)		Survivors (n=200)		All-cause deaths (n=70)		All-cause p value* ¹		S. aureus-attributable deaths (n=55)		Attributable p value* ²
	Number, % of all sites	Number, % of overall patients	Number, % of survivors	Number, % of all deaths	Number, % of survivors	Number, % of all deaths	Number, % of all deaths	Number, % of all deaths	Number, % of all deaths	Number, % of all deaths	Number, % of all deaths	Number, % of all deaths	
Pattern of disease													
Bacteraemia only, no identified site of infection													
1 identified site of infection													
>1 identified site of infection													
Blood culture positive													
Superficial abscesses (skin and soft tissue)													
Deep abscesses* ³													
Other skin and soft tissue infections* ⁴													
Bone and joint infections													
- Septic arthritis													
- Osteomyelitis													
- Diabetic foot infection													
Prosthetic material infections													
- Orthopaedic material* ⁵													
- Intravenous device* ⁶													
- Pacemaker													
- Meningitis related to ventriculostomy drain													
- Arteriovenous graft													
- Peritonitis from peritoneal dialysis													
Respiratory infections													
- Pneumonia													
- Empyema													

Endocarditis* ⁷	8 (3%)	8 (3%)	6 (3%)	2 (3%)	>0.999	2 (4%)	0.667
Other infections* ⁸	7 (3%)	7 (3%)	4 (2%)	3 (4%)	0.380	2 (4%)	0.634
Post-operative infections* ⁹	6 (2%)	6 (2%)	3 (2%)	3 (4%)	0.182	3 (5%)	0.101

Data are number (%).

*¹ p value for the comparison between all-cause deaths and survivors.

*² p value for the comparison between *S. aureus*-attributable deaths all other patients (survivors and other deaths combined).

*³ Site of deep abscesses were muscle (n=20), retroperitoneal space (n=7), parotid gland (n=7), liver (n=3), lung (n=2), epidural space (n=2), eye (n=2), oropharynx (n=2) and spleen (n=1).

*⁴ Other skin and soft tissue infections includes: necrotising fasciitis (n=9), bedsore(s) (n=6), pustules and carbuncles (n=5), infected wound from trauma (n=3), infected wound from tophi (n=2), gangrene (n=2), cellulitis (without other skin or soft tissue lesion) (n=2) and infection of exfoliated skin following a severe drug reaction (n=2).

*⁵ Orthopaedic material includes: internal fixation metalwork (n=8) and a hip replacement (n=1).

*⁶ Intravenous devices were peripheral cannulas (n=4), central catheters (n=3) and an umbilical catheter (n=1).

*⁷ Endocarditis from transthoracic echocardiographic evidence of vegetations (n=7); 1 case clinically but died prior to echocardiogram

*⁸ Other infections include: urinary tract infection (n=3), tenosynovitis (n=2), Lemierre's syndrome (n=1) and corneal ulcer (n=1)

*⁹ Post-operative infections include: mediastinitis (n=4; 3 following mitral valve replacement and 1 after coronary artery bypass graft), meningitis from infected bone flap surgical wound (n=1) and abdominal wound (n=1).

4.2.6 Drug resistance

MRSA accounted for 16% (n=42) of infections overall, increasing to 26% in those patients with bacteraemia. There was no significant difference in the MRSA rates between adults and children. No community-acquired MRSA was identified, the MRSA infections being defined as nosocomial (n=29, 69%), or non-nosocomial healthcare-associated (n=13, 31%). MSSA infections were predominantly community-acquired (84%, n=191), the remainder being nosocomial (n=23, 10%) or non-nosocomial healthcare-associated (n=14, 6%). MRSA infection was positively associated with all-cause death; 19/200 (10%) patients who survived were infected with MRSA compared with 23/70 (33%) of those who died ($p<0.001$) (Figure 4.1), and the association remained highly significant for attributable mortality ($p=0.001$).

4.2.7 Antibiotic therapy

Antibiotic therapy was prescribed to 269/270 patients, the single exception being a patient who was discharged from hospital prior to the culture results becoming available. The majority (n=253, 94%) of patients started antibiotics on or before the day their positive culture was taken. Of these, 220 patients (87%) received an empiric antibiotic that covered their infecting strain of *S. aureus* as judged by *in vitro* susceptibility testing. Of the remainder, 42 patients had a delay in starting antibiotic therapy to which the organism was susceptible (median delay 3 days, IQR 2-4 days), and 7 patients never received an effective antibiotic. A delay in receiving effective antibiotic therapy or never receiving effective therapy was significantly associated with an increased all-cause mortality (50% vs. 20%, $p<0.001$) (Figure 4.2) and attributable mortality ($p<0.001$).

4.2.8 Infectious source control

A procedure for infectious source control was performed in 184 patients (68%). This was associated with a significantly improved outcome: mortality with and without a procedure was 12% and 56%, respectively ($p<0.001$) (Figure 4.2). The procedures were: needle aspiration (n=10), incision and drainage (n=109), debridement (n=35), joint washout (n=8),

removal of prosthesis (n=7), thoractomy/ decortication (n=4), nephrectomy (n=3), laminectomy (n=2), chest drain (n=2), above knee amputation (n=1), fasciotomy (n=1), ethmoidectomy (n=1) and craniectomy with removal of infected bone flap (n=1).

4.2.9 Panton-Valentine Leukocidin

The infecting *S. aureus* isolates from 248 patients were available for PCR, of which 122 (49%) were positive for the genes encoding PVL, all of which were MSSA. The white cell count at the time of culture was not significantly different between PVL gene-positive and PVL gene-negative cases (median $15.30 \times 10^9/L$ vs. $14.69 \times 10^9/L$, respectively, $p=0.38$). The distribution of clinical presentations and outcome in relation to PVL status is shown in Table 4.3. PVL gene-positive isolates were significantly more likely to cause skin and soft tissue abscesses ($p<0.001$) and deep abscesses ($p=0.001$) than PVL gene-negative isolates. A significant negative association was observed between the presence of PVL genes and bacteraemia without a localising site of infection ($p<0.001$), prosthetic material infections ($p<0.001$) and other skin and soft tissue infections ($p=0.026$). Additionally, there was a highly significant negative association between the presence of PVL genes and all-cause mortality: mortality was 11% amongst PVL gene-positive cases and 39% in PVL gene-negative cases ($p<0.001$) (Figure 4.1) as well as *S. aureus*-attributable mortality ($p<0.001$). This association remained highly significant when adjusted for MRSA cases ($p=0.001$). The outcome from pneumonia was poor regardless of PVL gene carriage: mortality was 80% for PVL gene-positive cases and 83% for PVL gene-negative cases.

Figure 4.1: Association between all-cause mortality and methicillin-resistant *S. aureus* (MRSA) and Panton-Valentine Leukocidin (PVL).

Patients infected by MRSA had a greater all-cause mortality compared with patients infected by methicillin-susceptible *S. aureus* (MSSA) ($p<0.001$). Conversely, patients infected by PVL gene-positive *S. aureus* had a lower all-cause mortality compared with patients infected by PVL gene-negative *S. aureus* ($p<0.001$), an association that remained after adjustment for MRSA ($p=0.001$).

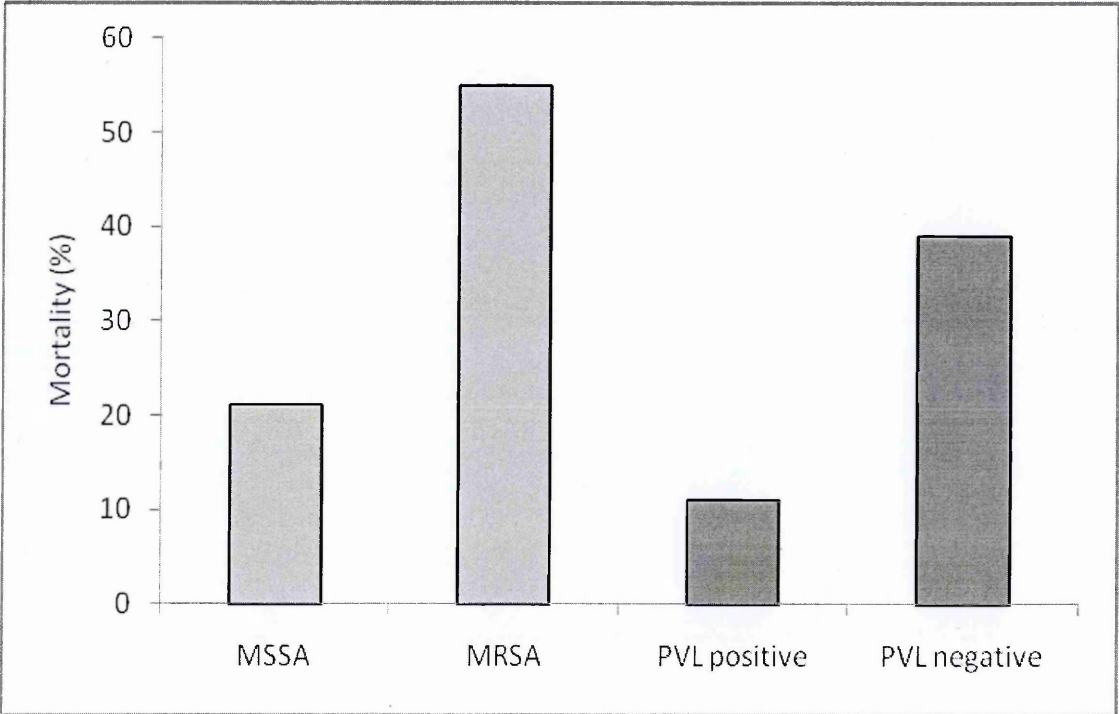
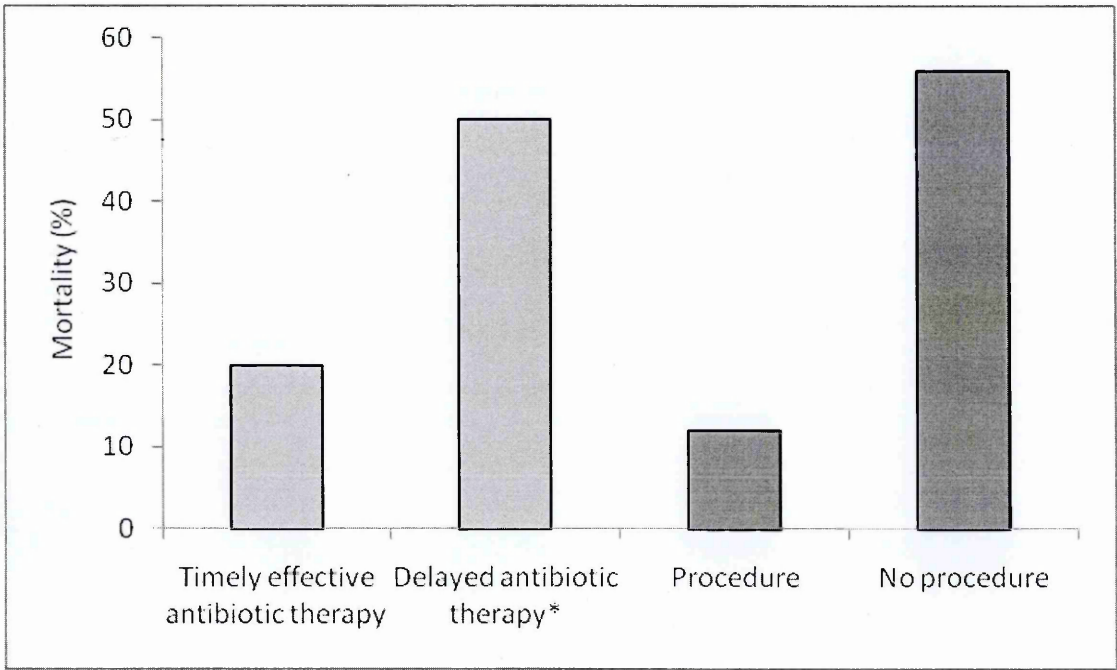


Figure 4.2: Association between all-cause mortality and timely effective antibiotic therapy and procedures for infectious source control.

Administration of an effective antibiotic on the same day as the positive culture was taken significantly reduced all-cause mortality ($p<0.001$), as did undergoing a procedure for infectious source control ($p<0.001$).



* Delayed antibiotic therapy included 7 patients who never received an effective antibiotic.

Table 4.3: Association between clinical presentation and presence of the gene encoding Panton-Valentine Leukocidin (PVL) in the infecting isolate for 248 patients with *S. aureus* infection

	Total no. of sites (n=250)	Total no. of patients (n=248)	pv/ positive (n=122)	pv/ negative (n=126)	p value ^{*1}
Clinical presentations					
Bacteraemia only, no identified site of infection	-	29 (12%)	3 (2%)	26 (21%)	<0.001
Superficial abscesses	86 (34%)	80 (32%)	60 (49%)	20 (16%)	<0.001
Deep abscesses	45 (18%)	43 (17%)	31 (25%)	12 (10%)	0.001
Other skin and soft tissue infections	29 (12%)	28 (11%)	8 (7%)	20 (16%)	0.026
Bone and joint infections	28 (11%)	25 (10%)	12 (10%)	13 (10%)	>0.999
Prosthetic material infections	24 (10%)	24 (10%)	2 (2%)	22 (17%)	<0.001
Respiratory infections	20 (8%)	19 (8%)	8 (7%)	11 (9%)	0.635
- Pneumonia ^{*2}	11 (4%)	11 (4%)	5 (4%)	6 (5%)	>0.999
Endocarditis	7 (3%)	7 (3%)	2 (2%)	5 (4%)	0.447
Other infections	7 (3%)	7 (3%)	5 (4%)	2 (2%)	0.275
Post-operative infections	4 (2%)	4 (2%)	0	4 (3%)	0.122
Outcome					
All-cause mortality		62 (25%)	13 (11%)	49 (39%)	<0.001
<i>S. aureus</i> -attributable mortality		48 (19%)	10 (8%)	38 (30%)	<0.001

Data are number (%). Denominator is the site of clinical presentation rather than patients.

^{*1} p value for the comparison between pv/-positive and pv/-negative cases.

^{*2} Subset of respiratory infections with pneumonia.

4.2.10 Risk factors for mortality

All variables in Tables 4.1 and 4.2 found to be significantly associated with all-cause and attributable mortality on univariable testing and with 5 or more events (deaths) together with the presence of PVL genes, were analysed further using multiple logistic regression to give the final models shown in Table 4.4. Cardiac, renal and lung disease were considered as separate variables for all-cause mortality, and cardiac and renal disease for attributable mortality. Only cardiac disease remained in each of the final models. Age, underlying cardiac disease and respiratory infection were positively associated with all-cause mortality, while one or more abscesses (deep and superficial combined) as the presenting clinical feature and a procedure for infectious source control were negatively associated with all-cause mortality. An analysis of *S. aureus*-attributable mortality demonstrated the same associations. Underlying cardiac disease and respiratory infection had the highest odd ratios for both all-cause mortality and *S. aureus*-attributable mortality.

Table 4.4: Significant risk factors for mortality from *S. aureus* infection from multiple logistic regression analysis

		Odds ratio (95% CI)* ¹	p value* ²
All-cause mortality			
Age		1.03 (1.01 – 1.05)	<0.001
Underlying cardiac disease		10.43 (2.96 – 36.70)	<0.001
Respiratory infection		6.46 (2.13 – 19.62)	0.001
Abscesses (superficial and deep combined)		0.13 (0.04 – 0.40)	<0.001
Procedure for infectious source control		0.22 (0.10-0.51)	<0.001
<i>S. aureus</i>-attributable mortality			
Age		1.03 (1.01 – 1.04)	0.001
Underlying cardiac disease		4.34 (1.43 – 13.19)	0.008
Respiratory infection		3.30 (1.16 – 9.41)	0.026
Abscesses (superficial and deep combined)		0.14 (0.04 – 0.53)	0.001
Procedure for infectious source control		0.17 (0.07 – 0.39)	<0.001

*¹ 95% confidence intervals

*² p value from Likelihood ratio test

4.3 Discussion

This prospective study generated much more detailed and informative data regarding invasive *S. aureus* disease in a resource-restricted tropical setting. The study adds to the evidence base that *S. aureus* is an important pathogen in developing countries in the tropics as it gives rise to considerable morbidity and mortality. The inclusion of cultures from all normally sterile sites rather than merely blood cultures yielded a more diverse range of clinical manifestations which mirrored those described from developed temperate countries. In particular, infections such as osteomyelitis, solid organ abscesses and endocarditis which had been absent or poorly represented in the retrospective study of *S. aureus* bacteraemia, accounted for a greater number of infections. The wider inclusion criteria also meant that there was no longer an apparent lack of investigations for pus collections and procedures for pus drainage.

The mortality rate for bacteraemia was again considerably higher than that reported from well resourced healthcare settings in Europe and the United States,^{52,161,479,483} with the majority of deaths being attributable to *S. aureus*. In the developed world, factors associated with poor outcome from *S. aureus* infection include increasing age,^{297,479} underlying co-morbidities,⁴⁸⁴ antimicrobial resistance,⁴⁸⁵ complicated (disseminated) bacteraemia,⁴⁸⁶ lack of source control²⁹⁷ including non-removal of intravenous catheters,²⁹⁸ under-dosing of penicillinase antibiotics for methicillin-susceptible *S. aureus* (MSSA),²⁹⁷ and delayed antibiotic therapy.¹⁸⁶ The relevance of these findings for the developing world has not yet been directly addressed in the published literature. This study demonstrated that many of these same factors were relevant for predicting mortality in a resource-restricted tropical setting; namely increasing age; underlying co-morbidities, in particular cardiac, renal and lung disease; methicillin resistance; lack of source control and delayed antibiotic therapy. Although in the retrospective study it was not possible to determine whether underlying co-morbidities were a confounding factor for the higher mortality seen with increasing age, the data from this study indicate that underlying co-morbidities are not a significant confounding factor. An awareness of those patient groups at high risk for death can have considerable clinical

utility in settings where healthcare facilities are available but limited and healthcare workers have a large patient caseload. Since the significant risk factors for death were data that are readily available without extra expense, identification of these risk factors is a practical option for those working in resource-restricted settings.

This hospital-based study conducted in provincial Thailand is relevant to the numerous developing countries with growing economies and expanding healthcare facilities, many of which are in South and East Asia, where it is possible to identify patients with suspected bacterial sepsis, administer empiric, broad-spectrum antibiotics, and drain pus collections. Practical, inexpensive solutions are needed to improve the outcome of bacterial sepsis in such developing country settings. A shorter time to the first dose of effective antibiotics has been related to a better outcome for a range of bacterial infectious diseases, including *S. aureus*, in the developed world,^{186,294,315} and held true in this study of *S. aureus* infection. Another low cost intervention is drainage of pus which is advocated as the standard of care elsewhere²⁹⁶ and would be predicted to reduce poor outcomes in developing country settings as well. Although both the low cost interventions of timely antibiotic therapy and drainage of pus collections were significantly associated with an improved outcome on univariate analysis, only procedures for infectious source control remained in the final model from multivariate analysis. This may be influenced by the fact that skin and soft tissue abscesses were the most common presentation and drainage of superficial pus collections can result in cure irrespective of antibiotic therapy.²⁹⁵ Antibiotic therapy in bacteraemic patients is analysed further in Chapter 5. This study indicates that identification of abscesses and drainage of pus is fundamental to sepsis control, and the use of available imaging to investigate patients with *S. aureus* sepsis and guide drainage procedures should be a priority. The higher mortality observed in our study for patients with bacteraemia in whom a site other than bloodstream was not identified is consistent with the higher mortality in patients who had a focus but no procedure for source control, and with the survival benefit for patients who underwent a procedure.

Nosocomial infection and drug resistance are the focus of enormous research efforts and expenditure in the developed world. In developing countries worldwide, these are generally poorly understood although there is wide variability. The importance of both nosocomial infection and drug resistance were highlighted in this study. Nearly one third of infections were nosocomial or non-nosocomial healthcare-associated, and patients in these groups had significantly higher mortality rates for both all-cause and *S. aureus*-attributable deaths. Infection with MRSA was either nosocomial or non-nosocomial healthcare-associated, and was independently associated with all-cause and attributable mortality. Given the importance of these findings, delineating the group of patients with non-nosocomial healthcare-associated infections was clearly a critical improvement in the prospective data. Those patients affected by healthcare-associated infection often have other risk factors for death such as prematurity, old age, underlying medical conditions or have undergone recent surgery and the high mortality rate is not surprising. However, healthcare-associated infections and infection-related deaths are potentially preventable and so there is a clear need for studies in resource-restricted tropical settings that evaluate the transmission of antibiotic resistance pathogens, such as MRSA, and low cost preventive interventions such as a hand hygiene campaign.

Nearly half of isolates causing *S. aureus* infection in patients presenting to our hospital were PVL gene-positive, which is significantly higher than that for previous reports for infecting isolates amongst blood culture and skin infection samples from Malaysia (5%),⁴⁸⁷ skin infections in Bangladesh (14.3%),⁴⁸⁸ and blood culture (2.1%) and soft tissue infection samples (38.9%) from the Netherlands.⁴⁸⁹ This may imply that PVL gene-positive strains are more likely to cause infection than PVL gene-negative isolates (that is, the rate of PVL gene-positivity is lower in carriage than invasive strains). This could either be a function of the presence and action of PVL per se, or could relate to one or more alternative genes associated with disease acquisition that is in genetic linkage with the genes encoding PVL.

The role of PVL as a putative virulence determinant is a hotly debated topic within the staphylococcal community, and this led me to examine the association between this

and both clinical presentations and outcome. The study design was robust in that all patients with *S. aureus* infection who required hospital treatment were recruited. Previous reports have been based on patient subsets determined by clinical presentation,²⁴⁸ culture sample type²⁵³ or by being sent to a reference laboratory.²⁶⁶ The single most important finding was that PVL gene-positive isolates were strongly associated with patient survival compared to PVL gene-negative isolates. This may be explained in large part by the fact that PVL gene-positive isolates were associated with skin and soft tissue abscesses, an association reported previously^{266,274,490} and a clinical manifestation associated with low morbidity. Community-acquired PVL-associated necrotising pneumonia affecting previously fit people is often fatal and has gained considerable notoriety. In this study there were 3 patients (1%) with PVL gene-positive, community-acquired pneumonia who were previously healthy individuals. None of these patients had haemoptysis, which is deemed a major diagnostic criterion for PVL-associated necrotising pneumonia,⁴⁹¹ although none of the prospective cases in the original paper by Gillet et al describing PVL-associated necrotising pneumonia were described as having had haemoptysis.²⁴⁸ Two out of 3 of these patients died, but this was in the context of a very high mortality of more than 80% for *S. aureus* pneumonia overall. Although further cohort studies of unselected patients are needed to determine the role of PVL as a virulence factor in other geographic settings, the current study provides no support for rapid PVL testing in this and other developing country settings, where the focus should firmly rest with clinical interventions that save lives.

Chapter 5. Prospective study of outcome from bacteraemia and impact of drug resistance on antibiotic prescribing

5.1 Chapter content

The prospective clinical study undertaken in the study hospital in northeast Thailand was based on the study of *S. aureus* bacteraemia carried out in the USA by Fowler et al.¹⁶¹ The similarity of design allowed for a comparison of *S. aureus* bacteraemia between a well-resourced temperate setting (USA) and a resource-restricted tropical setting (northeast Thailand). This chapter focuses on the subset of 98 patients with bacteraemia from the cohort of 270 prospectively identified patients with invasive *S. aureus* disease. The study methods were described in Chapter 2.

5.2 Results

5.2.1 Patient characteristics

Over the 1 year study period a total of 106 patients were identified with at least one clinically significant blood culture positive for a pure growth of *S. aureus*. Of these, 98 patients were recruited and 8 patients could not be studied either because they declined consent (n=6) or because they left the hospital for Lao PDR (across the Mekong River from Thailand) before the culture became positive and follow-up was not possible (n=2). The patient characteristics are shown in Table 5.1. The age of enrolled patients ranged from 1 day to 92 years (median 39 years). There were 61 (62%) adult and 37 paediatric patients (less than 18 years). There was a slight preponderance of males (58%) but this difference was not statistically significant. A history of one or more underlying chronic medical conditions was documented in 57 cases (58%), with cardiac disease accounting for the greatest proportion (20 cases).

Table 5.1: Patient characteristics

	All patients (n=98) <i>Number of patients (percent)</i>
Demographics	
Age (years), median (interquartile range)	39 (9 – 65)
Sex (male)	57 (58%)
Thai nationality	96 (98%)* ¹
Co-morbidities	
Prematurity/ Very low birth weight	5 (5%) (29% of those aged under 1 year)
Intravenous drug use	4 (4%)
Underlying medical conditions	57 (58%)
- Cardiac disease	20 (20%)
- Diabetes mellitus	12 (12%)
- Renal disease	11 (11%)
- Immunosuppression* ²	11 (11%)
- Lung disease	6 (6%)
Place of acquisition	
Community-acquired	44 (45%)
Nosocomial	40 (41%)
Non-nosocomial healthcare-associated	14 (14%)

*¹ Two patients were from Lao PDR

*² Immunosuppression from chemotherapy (n=4), untreated leukaemia (n=1), HIV (n=3, none of whom were on anti-retroviral therapy or any prophylactic antibiotics) or immunosuppressive medication including prednisolone >30mg/ day for >1 week (n=4; one of these additionally on chemotherapy)

5.2.2 Types of infection

A site of infection was identified in 59 (60%) patients. Of the 13 patients with more than 1 site of infection, 12 had 2 sites and 1 had 3 sites identified. There was a diverse spectrum of disease manifestations as shown in Table 5.2. The most common sites of infection were skin and soft tissue infection (18 sites, 25% of all sites) and pneumonia (12 sites, 16%).

5.2.3 Healthcare exposure

Over half of the infections (55%) were either nosocomial or non-nosocomial healthcare-associated (Table 5.1). Nosocomial infections were most common in those aged less than 1 year old, accounting for 94% of cases. Infections related to devices were the third most common site of infection, accounting for 14% of all sites. Additionally 4 patients had mediastinitis following thoracic surgery.

Table 5.2: Sites of infection

Types of disease	All patients (n=98) <i>Number of patients (percent)</i>
No identified site of infection	39 (40%)
1 identified site of infection	46 (47%)
>1 identified site of infection	13 (13%)
Sites of infection	All sites (n=73) <i>Number of sites (percent)</i>
Skin and soft tissue infection	18 (25%)
Pneumonia	12 (16%)
Device-related infection* ¹	10 (14%)
Endocarditis* ²	8 (11%)
Septic arthritis	7 (10%)
Post-operative mediastinitis* ³	4 (5%)
Other lung infection* ⁴	4 (5%)
Solid organ abscess* ⁵	3 (4%)
Osteomyelitis	2 (3%)
Pyomyositis	2 (3%)
Diabetic foot infection	2 (3%)
Urinary tract infection	1 (1%)

*¹ Intravenous catheters (central n=3, peripheral n=2, umbilical n=1), pacemakers (n=3) and arteriovenous graft (n=1)

*² Vegetations on transthoracic echocardiography (n=7); or strong clinical evidence in intravenous drug user (n=1)

*³ Following mitral valve replacement (n=3), or coronary artery bypass graft (n=1)

*⁴ Empyema (n=2), septic emboli to the lungs (n=1), lung abscesses (n=1)

*⁵ Liver (n=2), spleen (n=1)

5.2.4 Prevalence of endocarditis

Transthoracic echocardiography was performed in 49 out of 98 patients (50%). Although the aim of the study was to perform transthoracic echocardiography on all patients, the remaining 49 patients either were discharged or died before an echocardiogram could be performed, were too sick to be taken to the echocardiography room, had a contraindication to transthoracic echocardiography, or the echocardiography machine or trained personnel were not available. However, there were no significant differences between those who did and did not undergo echocardiography in terms of pre-disposing cardiac conditions, co-morbidities, immunocompromise, age or presence of prosthetic material (data not shown). Seven of the 49 patients (14%) undergoing transthoracic echocardiography had vegetations visualised. The affected heart valves were mitral (n=3), aortic (n=3) and tricuspid (n=1). Two patients had definite endocarditis and 5 had possible endocarditis by modified Duke criteria,⁴⁹² since taking 2 blood culture bottles is uncommon in the hospital. Among these 7 patients with endocarditis, 1 died. One additional patient with a history of injection drug use met clinical criteria for endocarditis, but died before echocardiography could be performed.

5.2.5 Persistent blood culture positivity

In addition to transthoracic echocardiography, the other study-specific investigation was taking a repeat blood culture within 48-96 hours of the first culture positive for *S. aureus*. A repeat blood culture was taken in 57 patients (58%, representing 79% of patients surviving to 4 days), of which 11 (19%) were still positive for *S. aureus*. After this time period, 56 further blood cultures were taken from 23 patients, which identified an additional 3 patients with persistently positive blood cultures. No vegetations were seen on transthoracic echocardiography performed on 11 of these 14 patients with persistently positive cultures. MRSA was responsible for persistently positive cultures significantly more often than MSSA infection (30% versus 8% respectively ($p=0.02$)). Eight of 14 patients (57%) were receiving antibiotic therapy to which the organism was susceptible *in vitro* at the time the repeat blood culture was taken.

5.2.6 Antibiotic resistance and therapy

MRSA accounted for 28% (n=27) of *S. aureus* bacteraemias, with no significant difference in rate between adults and children (26% versus 30% respectively; $p=0.82$). The MRSA cases were either nosocomial (n=21, 78%) or non-nosocomial healthcare-associated (n=6, 22%). Of the 71 MSSA cases, 44 (62%) were community-acquired, 8 (11%) were non-nosocomial healthcare-associated, and 19 (27%) were nosocomial. Although MRSA cases occurred throughout the hospital, there was a significant difference in MRSA rates on the intensive care units compared with the general wards: 57% versus 16%, respectively ($p<0.001$).

The impact of MRSA on effective prescribing of antibiotic therapy is summarised in Table 5.3. Patients infected with MSSA were more likely than patients infected with MRSA to receive an antibiotic to which the organism was susceptible before the culture results became available (67/71 (94%) versus 4/27 (15%) respectively, $p<0.001$). The median number of days to starting antibiotic therapy to which the organism was susceptible *in vitro* was 0 (IQR 0-0) days and 3 (IQR 2-4) days for MSSA and MRSA, respectively ($p<0.001$). Once culture results were available, 70 out of 73 survivors (96%) received an antibiotic that covered the infecting isolate. The median duration of parenteral treatment given to survivors from the day the first culture positive for *S. aureus* was taken was 17 (IQR 12-24) days, with no difference in duration for patients infected with MSSA versus MRSA ($p=0.26$).

All MRSA isolates were susceptible to vancomycin by E-test (minimum inhibitory concentration $\leq 2\mu\text{g/ml}$). Of the 17 patients with normal renal function who received vancomycin, 13 (76%) received adequate vancomycin doses by manufacturer's recommendations. Measurement of vancomycin drug levels was not available in the hospital. Antibiotic resistance patterns are summarised in Table 5.4. Over 90% of MRSA isolates were resistant to ciprofloxacin, erythromycin, gentamicin, netilmicin, tetracycline and trimethoprim-sulphamethoxazole, and 65% were resistant to clindamycin. Vancomycin and fusidic acid had the lowest rates of resistance.

Table 5.3: Impact of methicillin-resistant *S. aureus* (MRSA) on effective antibiotic prescribing

	All patients (n=98)	MSSA* ¹ (n=71)	MRSA* ¹ (n=27)	p value* ²
	<i>Number of patients (percent)</i>			
Pre-culture results				
Antibiotic therapy prescribed	93 (95%)	67 (94%)	26 (96%)	>0.99
Infecting strain of <i>S. aureus</i> susceptible to prescribed antibiotic	71 (72%)	67 (94%)	4 (15%)	<0.001
Optimal antibiotic therapy* ³	25 (26%)	23 (32%)	2 (7%)	0.01
Post-culture results*⁴	All patients (n=73)	MSSA (n=50)	MRSA (n=23)	
Antibiotic therapy prescribed	72 (99%)	49 (98%)	23 (100%)	>0.99
Infecting strain of <i>S. aureus</i> susceptible to prescribed antibiotic	70 (96%)	48 (96%)	22 (96%)	>0.99
Optimal antibiotic therapy* ³	53 (73%)	32 (64%)	21 (91%)	0.02

*¹ MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*

*² p value comparing MSSA and MRSA groups

*³ Optimal therapy defined as cloxacillin for MSSA infection and vancomycin for MRSA infection. Alternative therapy used included ceftriaxone, cefazolin, ceftazidime, augmentin and ampicillin combined with gentamicin.

*⁴ Denominator is patients who survived to day of culture result. A total of 25 patients (21 with MSSA and 4 with MRSA) died or were discharged moribund prior to culture results becoming available

Table 5.4: Antibiotic resistance rates for infecting isolates

Antibiotic	All isolates (n=81)	MSSA* ¹ (n=58)	MRSA* ¹ (n=23)
	Number of resistant isolates (%)		
Chloramphenicol	8 (10%)	4 (7%)	4 (17%)
Ciprofloxacin	22 (27%)	1 (2%)	21 (91%)
Clindamycin	16 (20%)	1 (2%)	15 (65%)
Erythromycin	25 (31%)	2 (3%)	23 (100%)
Fusidic acid	42 (52%)	39 (67%)	3 (13%)
Gentamicin	23 (28%)	0	23 (100%)
Mupirocin	7 (9%)	2 (3%)	5 (22%)
Netilmicin	21 (26%)	0	21 (91%)
Penicillin	80 (99%)	57 (98%)	23 (100%)
Rifampicin	8 (10%)	0	8 (35%)
Tetracycline	56 (69%)	34 (59%)	22 (96%)
Trimethoprim-sulphamethoxazole	22 (27%)	0	22 (96%)
Vancomycin	0	0	0

*¹ MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*

5.2.7 Mortality

The overall mortality rate at 12 weeks was 52% (n=51), and the *S. aureus* attributable mortality rate was 44% (n=43). In 1 case there was insufficient information to determine the cause of death. Most deaths (39/51, 76%) occurred in hospital or at home on the day of discharge, as relatives commonly take moribund patients home to die (n=12). *S. aureus* bacteraemia accounted for 1% of all in-patient deaths at Sappasithiprasong hospital during the study period.

Overall mortality increased significantly with age ($p<0.001$) (Figure 5.1). There was a significant difference between the overall mortality rate in children compared to adults (32% versus 64%, $p=0.003$). The association between overall mortality and increasing age remained highly significant when adjusted for underlying co-morbidities or immunosuppression ($p=0.001$). The mortality rates for MRSA and MSSA were 67% (18/27) and 46% (33/71), respectively ($p=0.11$). There was no significant difference in mortality between patients with and without identified sites of infection, (47% and 59% respectively, $p=0.31$). Mortality amongst patients with repeated blood cultures positive for *S. aureus* was 43% (6/14). The median number of days to death in those patients whose death was attributable to *S. aureus* was 3 (IQR, 1-6) days compared with 47 (IQR, 25-68) days in those with non-attributable deaths ($p<0.001$). Significant differences were noted on survival curve analysis of attributable mortality rates in adults and children ($p=0.01$) (Figure 5.2), and in the timing of death for *S. aureus* attributable and non-attributable deaths ($p=0.001$) (Figure 5.3). There was no difference in times to death comparing patients infected with MSSA versus MRSA (data not shown). At 12 weeks, 44 patients (45%) were cured, and 3 patients (3%) had unresolved infection (empyema thoracis, infected pacemaker, and osteomyelitis).

Figure 5.1: Age distribution and age-specific mortality rates and disease burden for 98 cases of *S. aureus* bacteraemia.

The burden of *S. aureus* bacteraemia is highest at the extremes of age, whilst mortality increased significantly with age ($p<0.001$), analysing age as a continuous variable.

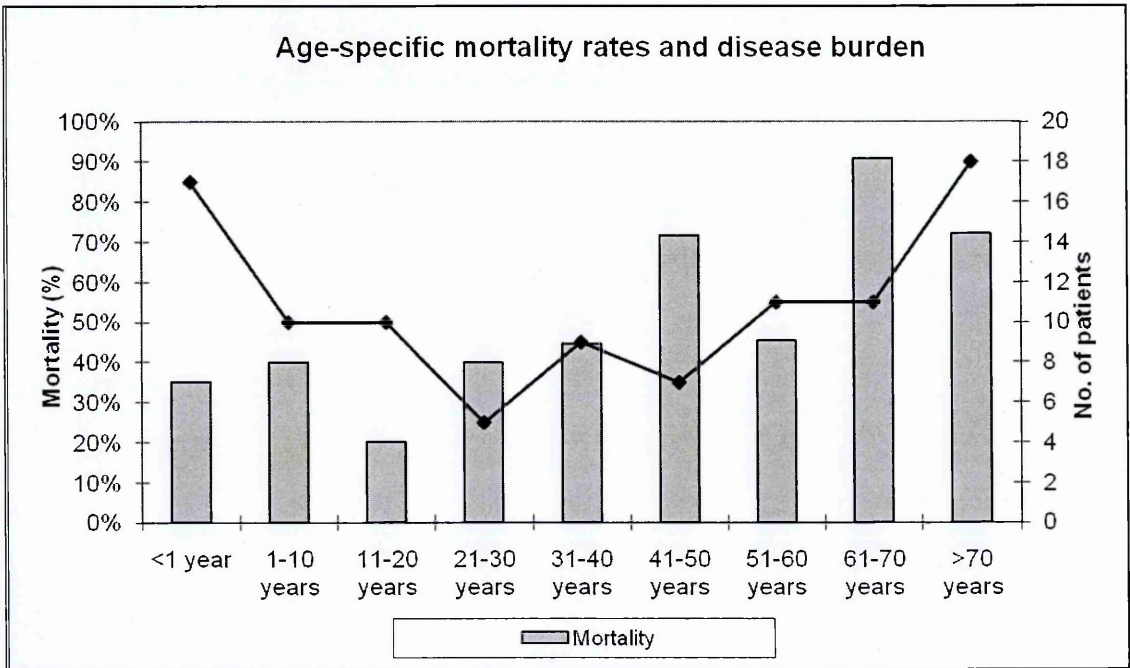


Figure 5.2: Kaplan-Meier survival curves comparing adult and paediatric patients with respect to *S. aureus*-attributable deaths.

Survival from *S. aureus* bacteraemia is worse in adults than in children. ($p=0.01$). Non-attributable deaths were censored.

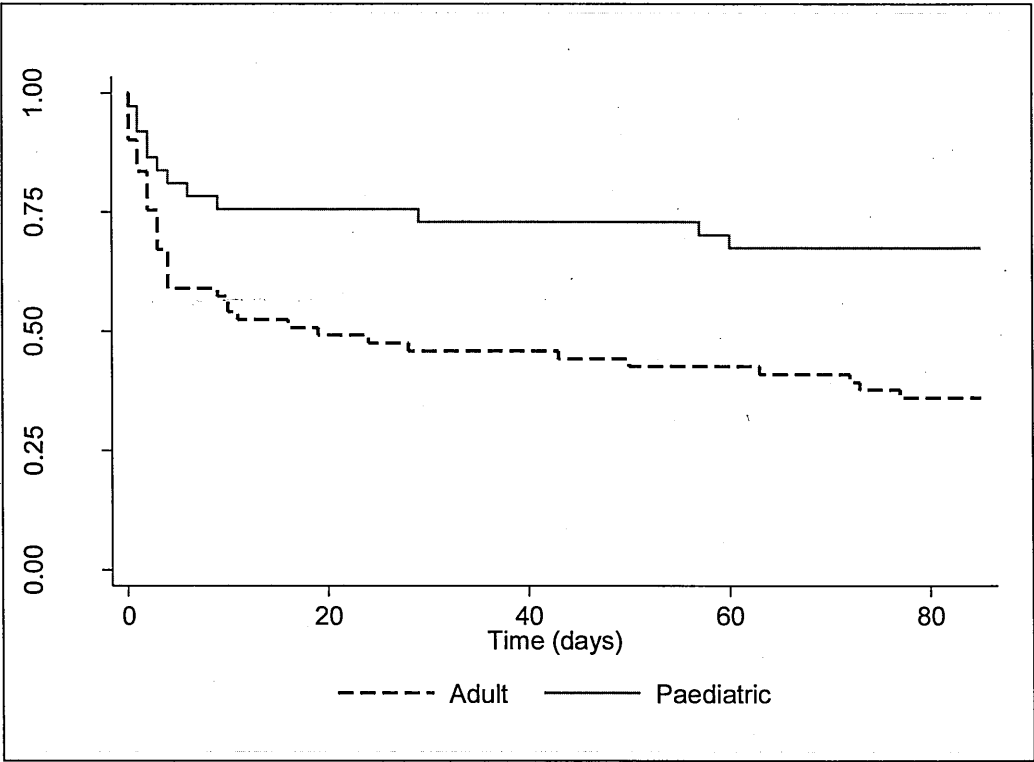
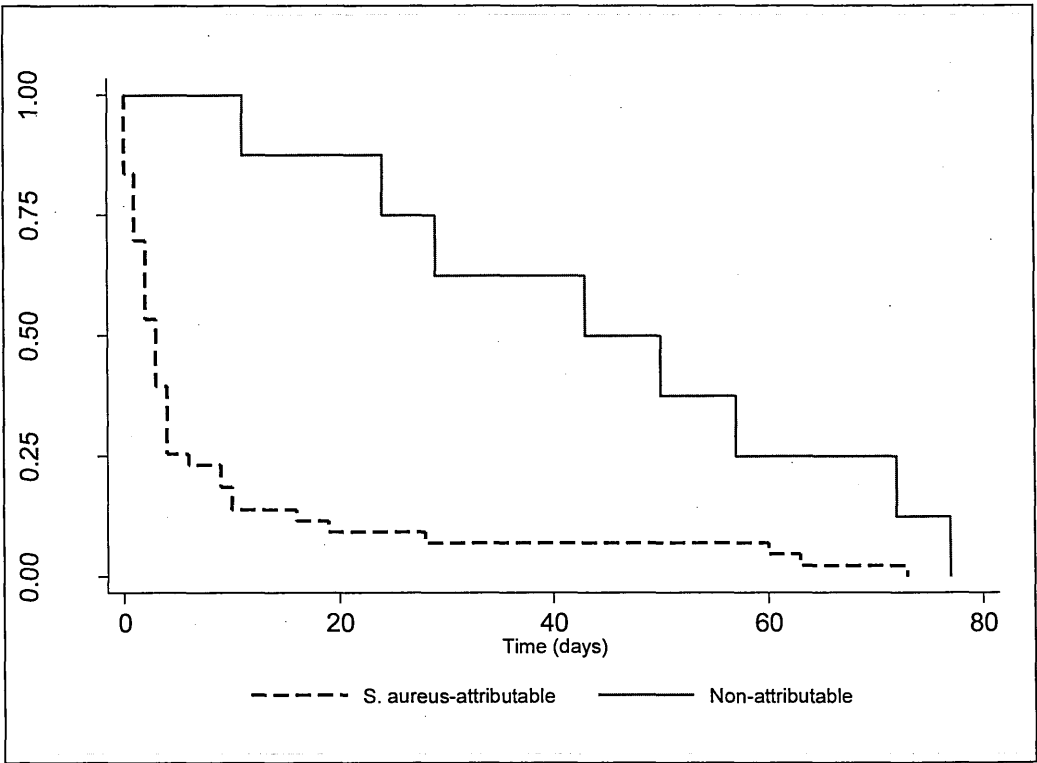


Figure 5.3: Kaplan-Meier survival curves comparing *S. aureus* attributable deaths and non-attributable deaths.

S. aureus attributable deaths occur more rapidly than non-attributable deaths ($p=0.001$).



5.3 Discussion

This prospective study reinforces the view that *S. aureus* bacteraemia is an important cause of morbidity and mortality in northeast Thailand. The all-cause and *S. aureus*-attributable mortality rates in this study, 52% and 44% respectively, were considerably higher than mortality rates for *S. aureus* bacteraemia generally reported from industrialised temperate countries.^{479,483,493} In comparison with the similarly designed study of *S. aureus* bacteraemia conducted in the USA by Fowler et al¹⁶¹, the mortality rates in Thailand were roughly double. Fowler et al found an all-cause and attributable mortality of 22% (157/724) and 12% (86/724), respectively, but they excluded deaths that occurred before culture results were available. The addition of these cases would have given an all-cause and *S. aureus*-attributable mortality rate of 33% and 23%, respectively.

These data on the importance of *S. aureus* bacteraemia as a cause of morbidity and mortality in a resource-restricted tropical setting are supported by published studies of all-cause bacteraemia in Asia, which have identified *S. aureus* as a major cause of bacteraemia, accounting for both community-acquired and hospital-acquired disease.^{8,494,495} Extrapolating further, if these data showing that *S. aureus* accounted for 1% of all in-patient deaths is representative of this populous region (half the world's population lives within a 2000 mile radius of northeast Thailand), then *S. aureus* would be a major contributor to preventable mortality worldwide. When this work was published it was the first published prospective study to focus on *S. aureus* bacteraemia in a resource-restricted tropical setting in Asia to my knowledge, having reviewed the literature. This was particularly noteworthy because papers describing all-cause bacteraemia in Asia rarely gave details by causative organism, resulting in an under-appreciation of the morbidity and mortality burden due to *S. aureus* in the tropics.

In this prospective study, *S. aureus* bacteraemia was most common at the extremes of age, a similar pattern to that described in a temperate industrialised country.^{52,53} The comparative study by Fowler et al¹⁶¹ did not include patients aged less than 18 years so the age structure of study participants cannot be compared. Additionally, because Sappasithiprasong Hospital is the regional hospital for the province with referrals

from clinics and hospitals in the province but also provides primary care services to a large local population, accurate estimates of disease incidence were not possible. Among the 17 patients under 1 year of age, 16 (94%) had hospital-acquired infections and nearly a third (29%) were premature or very low birth weight babies. This indicates that children less than 1 year of age who require multiple interventions and a prolonged stay in hospital are at particular risk of nosocomial *S. aureus* bacteraemia. A higher incidence in those aged under 1 year and the predominance of nosocomial infections in this age group has been described in Denmark.⁵³ The increase in number of cases and mortality with age also mirror the rise in co-morbidities with age, as noted in Denmark.⁵²

A broader range of clinical manifestations of *S. aureus* bacteraemia was seen compared with the retrospective study, as hypothesised given the increased detail obtainable with a prospective study. The findings mirrored more closely the diversity of clinical manifestations described for patients with *S. aureus* bacteraemia in temperate industrialised countries. Interestingly, the 2 most common manifestations (skin and soft tissue infection followed in frequency by pneumonia) were the same in the both the retrospective and prospective studies in spite of the greater diversity of manifestations in the latter. The study findings suggested that the burden of *S. aureus* disease in the tropics exceeded current perceptions, and demonstrated that serious invasive infections were common. This has an important bearing on antimicrobial therapy because management of deep infections and bacteraemia requires effective antimicrobial therapy whereas drainage of superficial pus collections can result in cure irrespective of antibiotic therapy.²⁹⁵ Over half of the patients had healthcare-associated infections and device-related infections were the third most common site of infection, suggesting that *S. aureus* bacteraemia in Thailand was strongly related to healthcare, thereby reflecting the situation in industrialised temperate countries.^{161,496,497}

The rapid deterioration and short median time to death (3 days) of patients dying from *S. aureus* septicaemia meant that echocardiograms could not be performed on many of the patients. However, the prevalence of echocardiographically-confirmed endocarditis of 14% (7/49) was comparable to that seen in industrialised temperate countries,^{161,498}

which has important implications for many tropical countries where restricted or delayed access to echocardiography is liable to be an issue. The study prevalence may have been an underestimate since those patients who died earlier may have been more likely to have endocarditis. A quarter of the patients with endocarditis died, which was at the lower end of the mortality range reported for *S. aureus* endocarditis (25-47%)⁴⁸² and may have been a further indication that a number of patients who died prior to echocardiography had undiagnosed endocarditis. The heart valves affected by endocarditis were predominantly left-sided, which was in keeping with the low number of intravenous drug users in the study. Half the patients with endocarditis were teenagers, which was a younger age group than typically seen in industrialised temperate countries^{482,499} but is described in other tropical countries,^{90,500} often as a result of rheumatic heart disease and uncorrected congenital heart disease. However, none of the patients with endocarditis in this study had known valvular abnormalities.

MRSA was responsible for nearly one third of cases of *S. aureus* bacteraemia, all of which were healthcare-associated. Although community-associated MRSA is a major problem elsewhere, there was no evidence found for this in the study hospital where a clone defined by multilocus sequence typing as sequence type 239 predominated,²²⁴ which has been strongly associated with healthcare acquisition. Although there were comprehensive hospital infection control guidelines in Sappasithiprasong hospital, implementing these was difficult due to a bed occupancy rate that often exceeded 100% and a lack of infrastructure, such as a scarcity of isolation rooms and only two hand wash basins on each of the general wards. Since over half the infections were healthcare-associated and MRSA accounted for nearly one third of *S. aureus* bacteraemias, clearly hospital infection control would be an important area for future clinical and microbiological research if improvements were to be made.

Significant delays in receiving effective antibiotic therapy were seen with MRSA bacteraemia compared with MSSA bacteraemia. Vancomycin was available in the study hospital but was not used in the empiric regimen for a patient with suspected bacterial sepsis unless they were already known to be MRSA positive. Delays in treating *S. aureus*

bacteraemia are known to have an adverse effect on outcome.¹⁸⁶ In light of the finding that 53% of *S. aureus* bacteraemia cases in the under 1 year old age group were MRSA and all MRSA strains were resistant to gentamicin, the routine usage of ampicillin plus gentamicin as empirical therapy for all children in that age group should be revised. Although alternative antibiotics to vancomycin may be appropriate therapy for MRSA, in the study setting the high rates of resistance found on susceptibility testing indicate that these alternatives would not be effective. A quarter of the patients with normal renal function were under-dosed for vancomycin, which is liable to lead to a poorer outcome. Monitoring of vancomycin levels was not possible at Sappasithiprasong Hospital, which may have led doctors to give lower doses to reduce the perceived risk of toxicity.

This study has demonstrated that *S. aureus* is a significant pathogen in northeast Thailand, with comparable clinical manifestations and a similar endocarditis prevalence but higher mortality than industrialised temperate countries. The factors contributing to this high death rate, such as the early management of sepsis, require further evaluation. The majority of infections were associated with exposure to healthcare settings and MRSA was associated with a considerable burden of disease and a high mortality. Revisions to the empiric prescribing practices to include MRSA therapy could be associated with significant benefit. An initiative to raise the profile of infection control is needed, together with work to characterise the burden and causes of hospital-acquired infections, including patient-to-patient transmission of MRSA, such that cost-effective solutions can be devised that are appropriate to this setting.

Chapter 6. Prospective study of the applicability of the ‘Surviving Sepsis Campaign’ guidelines in a resource-restricted setting

6.1 Chapter content

Supportive sepsis care is an essential aspect of management for severe infections if deaths are to be prevented. An evaluation of the high mortality rate of *S. aureus* bacteraemia found in the prospective study, as discussed in Chapter 5, needs to consider the role of supportive sepsis care. The ‘Surviving Sepsis Campaign’ international guidelines for the management of severe sepsis and septic shock were first published in 2004³³⁹ and were then updated in 2008.³⁴⁰ These guidelines describe best practice for clinical care in resource-rich settings and have been very influential in improving outcomes from sepsis in the developed world. The extent to which it would be possible to follow these guidelines in a resource-restricted tropical setting has not previously been determined at the time of writing. This chapter assesses the feasibility of determining the presence of severe sepsis and septic shock, and the extent to which the management of patients with severe sepsis and septic shock caused by *S. aureus* bacteraemia in a large provincial hospital in Thailand could follow the ‘Surviving Sepsis Campaign’ guidelines. The study hospital had not adopted the guidelines prior to or during the study.

6.2 Results

6.2.1 Hospital resources

The study hospital has 1,100 beds and serves a population of around 2 million people living in the province of Ubon Ratchathani, where the major occupation is rice farming. The hospital facilities include a range of routine laboratory and radiology services including a CT scanner. General medical, surgical and orthopaedic wards are predominantly open-plan and contain around 30 beds per ward, increasing up to 60 beds per ward in response to demand. There are 13 separate intensive care units (ICU) (each with between 8 and 14 beds), of which 4 are for paediatric patients and 9 are for adults. The registered nurse to patient ratio on the general wards is approximately 1:8 and is

1:2.5 - 1:3.5 on the ICUs. Mechanical ventilation is available in these ICUs; children and adults may be ventilated by electrically powered multi-function ventilators (e.g. Bear Cub, Infant Star, or Bennett). Patients on the general ward are commonly ventilated by older gas driven ventilators (Bird Mark 7).

6.2.2 Sepsis definitions

Children were defined as 18 years of age or less in keeping with paediatric sepsis classification.³⁰¹ The standard sepsis definitions²⁹⁹⁻³⁰¹ were adopted: sepsis was systemic inflammatory response syndrome (SIRS) associated with infection, which in this study was clinically significant *S. aureus* bacteraemia; severe sepsis was SIRS with organ dysfunction, sepsis-induced hypotension, or hypoperfusion, as denoted by oliguria or acidosis; septic shock was SIRS with sepsis-induced hypotension and/or the use of vasoactive drugs. As described in Chapter 5, there were 98 patients with *S. aureus* bacteraemia enrolled in the study, of whom 88 patients (90%) met the standard definition for sepsis (Tables 6.1 and 6.2). From the available data and adapted criteria shown in Tables 6.1 and 6.2, a total of 73 patients (49 adults) had severe sepsis, of whom 48 patients (31 adults) had septic shock. The usual inclusion of hypotension despite adequate fluid resuscitation was not possible because of the format of documentation by treating physicians. Although arterial blood gases were available for 15 patients it was not possible to calculate the fraction of inspired oxygen, used to determine respiratory dysfunction, as inspired oxygen levels were not recorded.

Table 6.1: Investigations to determine systemic inflammatory response syndrome (SIRS) and organ dysfunction over the 3-day management period in adult patients

SIRS^{*1}	Tests/ data required	Patients with data number (% of total)	Patients with dysfunction number (% of patients with data)	Criteria used (based on standard definitions^{299,300)})
Adults (n=61)				
SIRS	Bedside observations	61 (100%)	61 (100%)	Temperature >38°C or <36°C; heart rate >90 beats per minute; respiratory rate >20 breaths per minute
	White cell count	60 (98%)	37 (62%)	White cell count <4 x10 ⁹ /L, >12 x10 ⁹ /L or >10% immature band forms
		59 adults (97%) fulfilled SIRS		Satisfied 2 out of 4 of criteria listed above
Organ dysfunction	Tests/ data required	SIRS patients with data number (% of total)	SIRS patients with dysfunction number (% of patients with data)	Criteria used (based on standard definitions^{299,300,501)})
Adults with SIRS (n=59)				
Cardiovascular	Blood pressure	57 (97%)	31 (54%)	Systolic blood pressure <90mmHg
	Vasoactive drug use	59 (100%)	19 (32%)	Dopamine >5µg/kg/min, or dobutamine, epinephrine or norepinephrine at any dose
Hypoperfusion	Oliguria	35 (59%)	13 (37%)	Urine output <500ml per 24 hours
	Acidosis	49 (83%)	22 (45%)	Total venous carbon dioxide <20mmol/L
Respiratory	Ventilator use	59 (100%)	24 (41%)	Mechanical ventilator used
Haematological	Platelet count	58 (98%)	15 (26%)	Platelets <100 x10 ⁹ /L
Renal	Creatinine	57 (97%)	25 (44%)	Creatinine ≥2mg/dL (≥177µmol/L)
Hepatic	Bilirubin	29 (49%)	7 (24%)	Bilirubin ≥2mg/dL (≥34µmol/L)
Neurological	Glasgow Coma Score	0	-	Glasgow Coma Score ≤12

^{*1} Systemic inflammatory response syndrome

Table 6.2: Investigations to determine systemic inflammatory response syndrome (SIRS) and organ dysfunction over the 3-day management period in paediatric patients

SIRS^{*1}	Tests/ data required	Patients with data number (% of total)	Patients with dysfunction number (% of patients with data)	Criteria used (based on standard definitions³⁰¹)
Children (n=37)				
SIRS	Bedside observations	37 (100%)	37 (100%)	Temperature >38.5°C or <36°C; age-specific cut-offs for heart rate and respiratory rate
	White cell count	36 (97%)	18 (50%)	Age-specific cut-offs for white cell count
		29 children (78%) fulfilled SIRS		Satisfied 2 out of 4 of criteria listed above, of which 1 must be either temperature or white cell count
Organ dysfunction	Tests/ data required	SIRS patients with data number (% of total)	SIRS patients with dysfunction number (% of patients with data)	Criteria used (based on standard definitions³⁰¹)
Children with SIRS (n=29)				
Cardiovascular	Blood pressure	23 (79%)	17 (74%)	Age-specific cut-offs for blood pressure
	Vasoactive drug use	29 (100%)	7 (24%)	Dopamine >5µg/kg/min, or dobutamine, epinephrine or norepinephrine at any dose
Hypoperfusion	Oliguria	10 (34%)	3 (30%)	Urine output <12ml/kg per 24 hours
	Acidosis	22 (76%)	11 (50%)	Total venous carbon dioxide <20mmol/L
Respiratory	Ventilator use	29 (100%)	12 (41%)	Mechanical ventilator used
Haematological	Platelet count	29 (100%)	8 (28%)	Platelets <80 x10 ⁹ /L
	Clotting	9 (31%)	2 (22%)	International normalised ratio >2
Renal	Creatinine	26 (90%)	2 (8%)	Creatinine ≥2 times upper limit of normal for age
Hepatic	Bilirubin	15 (52%)	2 (13%)	Bilirubin ≥4mg /dL (not applicable for newborn)
	Alanine transaminase	12 (41%)	3 (25%)	Alanine transaminase 2 times upper limit of normal for age
Neurological	Glasgow Coma Score	0	-	Glasgow Coma Score ≤11

^{*1} Systemic inflammatory response syndrome

6.2.3 Patient characteristics

Since the 'Surviving Sepsis Campaign' guidelines target severe sepsis, the characteristics of the 73 patients with severe sepsis are considered in detail and shown in Table 6.3. The age ranged from 2 days to 92 years (median 48 years); 24 (33%) patients were children. A history of any chronic underlying medical condition was documented in 47 cases (64%), with cardiac disease accounting for the greatest proportion. An identified site of infection, beyond bacteraemia, was found in 46 (63%) patients. MRSA was responsible for infection in 19 patients (26%).

6.2.4 Mortality

At 12-week follow-up, the mortality rates from severe staphylococcal sepsis overall or for the subset with septic shock were 58% and 65%, respectively. The majority of deaths (86%) occurred in hospital or at home on the day of discharge, as it is common for moribund patients to be taken home to die by their family. The 12-week mortality rate for severe sepsis from MRSA was 68% compared with 54% for MSSA ($p=0.30$). The median time to death for patients with severe sepsis was 3 days (IQR 1-9), and for the subset with septic shock was 2 days (IQR 1-4).

Table 6.3: Characteristics of 73 patients with severe staphylococcal sepsis

	Patients (n=73)
	<i>number of patients (%)</i>
Demographics	
Age (years)	48 (12 – 66)* ¹
Sex (male)	57 (58%)
Thai nationality* ²	72 (99%)
Co-morbidities	
Prematurity/ Very low birth weight	3 (4)
Underlying medical conditions* ³	47 (64)
- Cardiac disease* ⁴	17 (23)
- Renal disease* ⁵	10 (14)
- Diabetes mellitus	9 (12)
- Immunosuppression* ⁶	7 (10)
- Lung disease* ⁷	4 (5)
Source and sensitivities	
Community-acquired disease	35 (48)
Healthcare-associated disease	10 (14)
Hospital-acquired disease	28 (38)
MRSA* ⁸	19 (26)
Types of disease	
0 identified sites of infection (bacteraemia alone)	27 (37)
1 identified site of infection, beyond bacteraemia	36 (49)
More than 1 identified site of infection, beyond bacteraemia	10 (14)
Sites of infection*⁹	All sites (n=57)
	<i>number of sites (percent)</i>
Skin and soft tissue infections	14 (25)
Pneumonia	10 (18)
Device-related infections* ¹⁰	8 (14)
Endocarditis* ¹¹	5 (9)
Septic arthritis	5 (9)
Post-operative mediastinitis* ¹²	3 (5)
Other lung infections* ¹³	3 (5)
Solid organ abscess* ¹⁴	3 (5)
Osteomyelitis	2 (4)
Pyomyositis	2 (4)
Diabetic foot infections	1 (2)
Urinary tract infection	1 (2)

*¹ Median (interquartile range)

*² The one non-Thai patient was from Lao PDR

*³ Past medical history of any underlying chronic medical conditions reported by the patient and or relative, or recorded in the medical notes

*⁴ Cardiac disease includes congenital heart disease (n=3), valvular heart disease (more than mild impairment) including rheumatic heart disease (n=7), ischaemic heart disease (n=4) and arrhythmias including heart block requiring a pacemaker (n=3).

*⁵ Renal disease incorporates end stage renal failure on chronic hemodialysis (n=2) and chronic renal failure secondary to diabetes mellitus (n=5), multiple myeloma (n=1), systemic lupus erythematosus (n=1) and unknown aetiology (n=1).

*⁶ Immunosuppression from chemotherapy (n=4), untreated leukaemia (n=1), HIV (n=1, not on anti-retroviral therapy) or immunosuppressive medication including prednisolone more than 30mg per day for more than 1 week (n=2, including 1 patient also on chemotherapy).

*⁷ Lung disease comprises previous tuberculosis (n=2), previous empyema (n=1) and a long term tracheostomy (n=1).

*⁸ Methicillin-resistant *S. aureus*

*⁹ Since some patients had more than 1 site of infection, the denominator for this section is the number of sites (n=57)

*¹⁰ Device-related infections were intravenous catheters (n=4; central n=2, peripheral n=1, umbilical n=1), pacemakers (n=3) and an arteriovenous graft (n=1)

*¹¹ Endocarditis from transthoracic echocardiographic evidence of vegetations (n=5)

*¹² Post-operative following mitral valve replacement (n=3)

*¹³ Other lung infections included empyema (n=1), septic emboli to the lungs (n=1) and lung abscesses (n=1)

*¹³ Solid organ abscesses were liver (n=2) and spleen (n=1)

6.2.5 Sepsis management

Overall, 27 of 73 patients (37%) received care in an ICU and the remainder were treated on a general ward; 13 children (54%) and 14 adults (29%) were treated in an ICU ($p=0.04$). The ICUs ran at full occupancy and access for adults was strongly influenced by bed availability. Of the patients who were admitted to an ICU, 21 (78%) had septic shock and 19 died (70%), in comparison to those patients cared for only on a general ward ($n=46$) of whom 27 (59%) had septic shock and 23 died (50%).

The extent to which the sepsis management of the 73 patients with severe sepsis or septic shock can be assessed against the 'Surviving Sepsis Campaign' guidelines is provided in Table 6.4. The details of patient management during the first 3 days after the first positive culture was taken are discussed below in a style that follows the format of Tables 3, 4, and 5 in the 2008 'Surviving Sepsis Campaign' guidelines.³⁴⁰

Table 6.4: Summary of sepsis management

	Severe sepsis, all patients (n=73)	Severe sepsis, without septic shock (n=25)	Septic shock (n=48)	'Surviving Sepsis Campaign' guidelines ³⁴⁰
	number of patients (%)			
Antibiotic therapy				
Pre-culture covers ^{*1}	54 (74)	18 (72)	36 (75)	Begin broad-spectrum antibiotic therapy within first hour. Re-assess regimen on basis of culture results and narrow coverage
Post-culture covers ^{*2}	49 (98) (49 out of 50)	21 (95) (21 out of 22)	28 (100) (28 out of 28)	
Cardiovascular				Begin fluid resuscitation immediately. Use fluid challenge technique (1L crystalloids or 300-500mls colloids over 30 mins)
Documented fluid bolus	19 (26)	0	19 (40)	Give vasopressors via central catheter; insert arterial catheter to measure mean arterial pressure
Central access ^{*3}	12 (16)	2 (8)	10 (21)	Measure as resuscitation goal
CVP readings ^{*4}	6 (8)	0	6 (13)	Measure urine output hourly as resuscitation goal
Fluid balance record	53 (73)	16 (64)	37 (77)	
Urinary catheterisation	37 (51)	6 (24)	31 (65)	
Vasoactive drug use (vasopressors)	29 (40)	1 (4) ^{*5}	28 (58)	Norepinephrine and dopamine centrally administered are initial vasopressors of choice in adults. Dopamine first line in children.
Dobutamine (inotrope)	7 (10)	0	7 (15)	Consider if venous oxygen saturation target not achieved
Red blood cell transfusion	23 (32)	6 (24)	17 (35)	Transfuse if haemoglobin less than 7mg/dL, target 9mg/dL
Respiratory				
Oxygen saturation monitoring only	26 (36)	6 (24)	20 (42)	Regular arterial blood gas monitoring to assess partial pressures of oxygen and carbon dioxide
Arterial blood gas monitoring (at least 1 value)	15 (21)	2 (8)	13 (27)	
Supplemental oxygen if not ventilated ^{*6}	20 (54) (20 out of 37)	9 (45) (9 out of 20)	11 (65) (11 out of 17)	To achieve optimal oxygenation
Ventilated	36 (49)	5 (20)	31 (65)	Use lung-protective strategies (controlling tidal volumes and airway pressures)

- *¹ Antibiotic received to which the strain of *S. aureus* grown was susceptible based on laboratory testing.
- *² Post-culture results: the denominator is the number of patients who survived for their culture results to be available. Antibiotic received to which the strain of *S. aureus* grown was susceptible based on laboratory testing.
- *³ Central access included central line or cut down in antecubital fossa.
- *⁴ Central venous pressure
- *⁵ Dopamine dose less than 5µg per kilogram per minute cut-off for defining septic shock
- *⁶ The denominator is the number of patients who were not ventilated.

6.2.5.1 Initial resuscitation and infection issues

(i) Initial resuscitation. Since patient recruitment followed blood culture results, initial resuscitation data was collected retrospectively. Additionally, given that the 'Surviving Sepsis Campaign' guidelines had not been adopted by the hospital, documented timing of initial resuscitation measures was not made.

(ii) Diagnosis and isolating the pathogen causing the episode of sepsis. The recruitment criteria of *S. aureus* bacteraemia meant all study patients had at least one blood culture taken. Two or more blood cultures were acquired in 10 of 73 patients during initial assessment. Almost all patients (96%) had radiological imaging performed.

(iii) Antibiotic therapy. A broad-spectrum intravenous antibiotic was received by 66 patients (90%) on the day that the first positive blood culture was taken, and all but one of the remainder received a broad-spectrum antibiotic 24 hours or more later. The exact timing of the initial antibiotic dose was not recorded so no assessment can be made of the proportion of patients receiving their initial antibiotic within the first hour after recognition of severe sepsis. The infecting isolate was susceptible to the empiric antibiotic used in 54 cases, but not in 15 cases infected with MRSA. Once culture results were available, almost all patients who were still alive (49 out of 50, 98%) received an antibiotic that covered their strain of *S. aureus*. Of the 12 patients with normal renal function receiving vancomycin, 8 (67%) received adequate vancomycin doses by manufacturer's recommendations. Serum vancomycin levels were not tested because this assay was not available.

(iv) Source identification and control. A specific anatomical site of infection was identified in 46 patients, of whom 15 patients (33%) had a procedure contributing to infectious source control (abscess drainage 6, debridement 5, joint wash-out 1, fasciotomy 1, above knee amputation 1, chest drain insertion 1). A further 5 patients had a potentially drainable collection of pus identified or infected prosthetic material but did not undergo a procedure as this was deemed unfeasible.

6.2.5.2 Haemodynamic support and adjunctive therapy

(i) Fluid therapy. A documented fluid challenge using crystalloids was received by 19 patients (26%), all of whom had septic shock. The volume given ranged from 150 to 250mls in adults. Peripheral intravenous catheters alone were used in 61 of 73 patients (84%), cut-downs in 8 patients (7 adults and 1 child) and centrally placed catheters in 4 patients (all children). Central venous pressure (CVP) readings were recorded in 6 patients with cut-downs, all of whom had septic shock. A urinary catheter was inserted in 37 patients (51%).

(ii) Vasopressors and inotropic therapy. Invasive monitoring of arterial pressure by an intra-arterial catheter was only possible in the cardiothoracic wards, and none of the patients in this study had this performed. All blood pressure measurements were made using cuffs. One or more vasoactive drugs were used in 28 out of 48 patients (58%) with septic shock and dopamine was most often used as the first line agent. The majority of patients receiving vasopressors or inotropes (69%) received the drug through a peripheral line.

(iii) Steroids and activated protein C. Steroids were not used in the context of sepsis, and recombinant human activated protein C was not available.

6.2.5.3 Other supportive therapy of severe sepsis

(i) Blood product administration. Red blood cell transfusions were given in response to lowered haemoglobin but were targeted to a haemoglobin greater than 9mg/dl. Since central or mixed venous saturations were not measured, red blood cell transfusions were not used to achieve a haematocrit of 30% in order to reach the saturations target.

(ii) Mechanical ventilation. A total of 36 out of 73 patients (49%) were ventilated. Of these, 22 (61%) patients were ventilated using the Bird Mark 7 model (16 on a general ward, 6 in an ICU) of whom 2 (9%) survived (both in an ICU), and 14 were ventilated using electrically powered mechanical ventilators, of whom 4 (29%) survived (all in an ICU). At least one arterial blood gas was recorded in 12 of the 36 patients who were ventilated,

while pulse oximetry measurements alone were used in 18 of these patients.

Supplemental oxygen was widely available.

(iii) Sedation, analgesia and neuromuscular blockade in sepsis. These are not part of routine care in adults or children. Sedation was used in children who did not otherwise tolerate mechanical ventilation. Adults ventilated on the general ward did not receive sedation.

(iv) Glucose control. Blood sugar monitoring was performed in 38 out of 73 patients (52%). Blood sugar control was achieved by intermittent point-of-care testing of capillary blood glucose and subcutaneous insulin.

(v) Renal replacement therapy. None of the patients with acute renal failure (n=18) or an additional 16 patients who were acidotic received any form of dialysis because in the setting of sepsis these were not indications for acute dialysis in the hospital.

(vi) Deep vein thrombosis prophylaxis and stress ulcer prophylaxis. These were not part of routine care and were not given.

6.3 Discussion

The burden of sepsis in developing countries is massive but grossly understudied in comparison to developed parts of the world. This study of severe staphylococcal sepsis is among the first to quantify prospectively the management and outcomes of patients with sepsis in a developing country setting. The mortality rate in this study is double the all-cause severe sepsis mortality rates reported from developed countries.⁵⁰²⁻⁵⁰⁵ This emphasises the need for concerted efforts to improve sepsis care in developing regions of the world. With only slight modifications to the standard criteria for determining organ dysfunction, it was feasible to ascertain the proportion of patients with severe sepsis and septic shock within the current resource capability of the hospital. A hospital policy to routinely document inspired oxygen levels for those patients having arterial blood gases samples taken, and Glasgow Coma Scores in sick patients would further improve the hospital physicians' ability to detect organ dysfunction. This is fundamental because

recognition of sepsis and its severity is an essential prerequisite to activating the appropriate sepsis management required.

Many basic resources are available in developing countries for the management of patients with severe sepsis and septic shock. Microbiology laboratory facilities are critical because knowledge of the causative organism facilitates the utilisation of disease-specific measures and antimicrobial therapy can be tailored according to the susceptibility profile. The choice of empiric antibiotic therapy covered *S. aureus* less than 75% of the time, although this largely reflected the hospital policy to only prescribe vancomycin for culture-confirmed MRSA. The majority of patients had radiological imaging performed and 75% of those with a pus collection identified underwent a procedure for infectious source control. The guidelines caution that investigations to obtain a diagnosis and infectious source control procedures should only be carried out when safe to do so, in particular since investigations and procedures that require transporting the patient away from an ICU have considerable risks even in developed countries. Many of the patient cohort were critically ill which hampered the ability of physicians to fully work up their patients. An important limitation of this study is that the patient cohort was biased towards those with potential disease-specific measures who would be predicted to have a better outcome by examining only staphylococcal sepsis as opposed to all-cause sepsis (which includes patients without an identified causative organism).

Certain more complex sepsis management therapies such as invasive haemodynamic monitoring, ventilation and dialysis are available in some developing countries. Fluid resuscitation in septic patients is challenging, even in developed countries with widespread use of invasive haemodynamic monitoring. Crystalloid intravenous fluids were widely available in the hospital, but patients with sepsis including those with septic shock received low volumes of fluids. This may have been due to concerns about precipitating volume overload because bedside observations and fluid balance records were the mainstay of assessing volume status, and acute dialysis in the context of sepsis was not available. Respiratory failure was often managed with mechanical ventilation. However, outcomes following institution of mechanical ventilation were uniformly poor. In

particular, all patients ventilated on the general ward died. Mechanical ventilation may simply serve as a marker for critically ill patients. This strongly suggests that focussing sepsis management on the underlying disease process is essential to prevent a progression towards respiratory failure. Although some more complex interventions may be available in developing country settings, if their use is not associated with a survival benefit, as in the case of mechanical ventilation on the general ward in this study, then their continued use should be carefully considered. Further study of the utility and cost-benefit of specific therapies that are more complex and expensive in these settings is needed.

It is likely that these findings would be reproduced in a large number of medical facilities throughout the developing world. Thus, there is an urgent need to develop methods to optimise sepsis care within the existing framework and resources of such settings. A workable strategy should focus on the basic elements of sepsis care, namely appropriate sampling for diagnostic microbiology, rapid antibiotic administration, adequate infusion of intravenous fluids and control of the infectious source. These core elements are likely to comprise much of the benefit in the treatment of sepsis. Initiating such a strategy is within the capability of large numbers of hospitals throughout the world, can be implemented without prohibitive expense, and has the potential to save many thousands of lives per year. Such an approach would if successfully implemented represent an important step for global health and a move away from the current narrow focus on care of patients in developed countries. Efforts to raise the profile of sepsis have been successful in developed countries but there remains a pressing need for both education of healthcare providers and comprehensive research into sepsis in developing countries. These endeavours are the next essential components of a strategy to tackle the mortality burden of sepsis worldwide.

Chapter 7. Methicillin-resistant *S. aureus* (MRSA) carriage and transmission study results

7.1 Chapter content

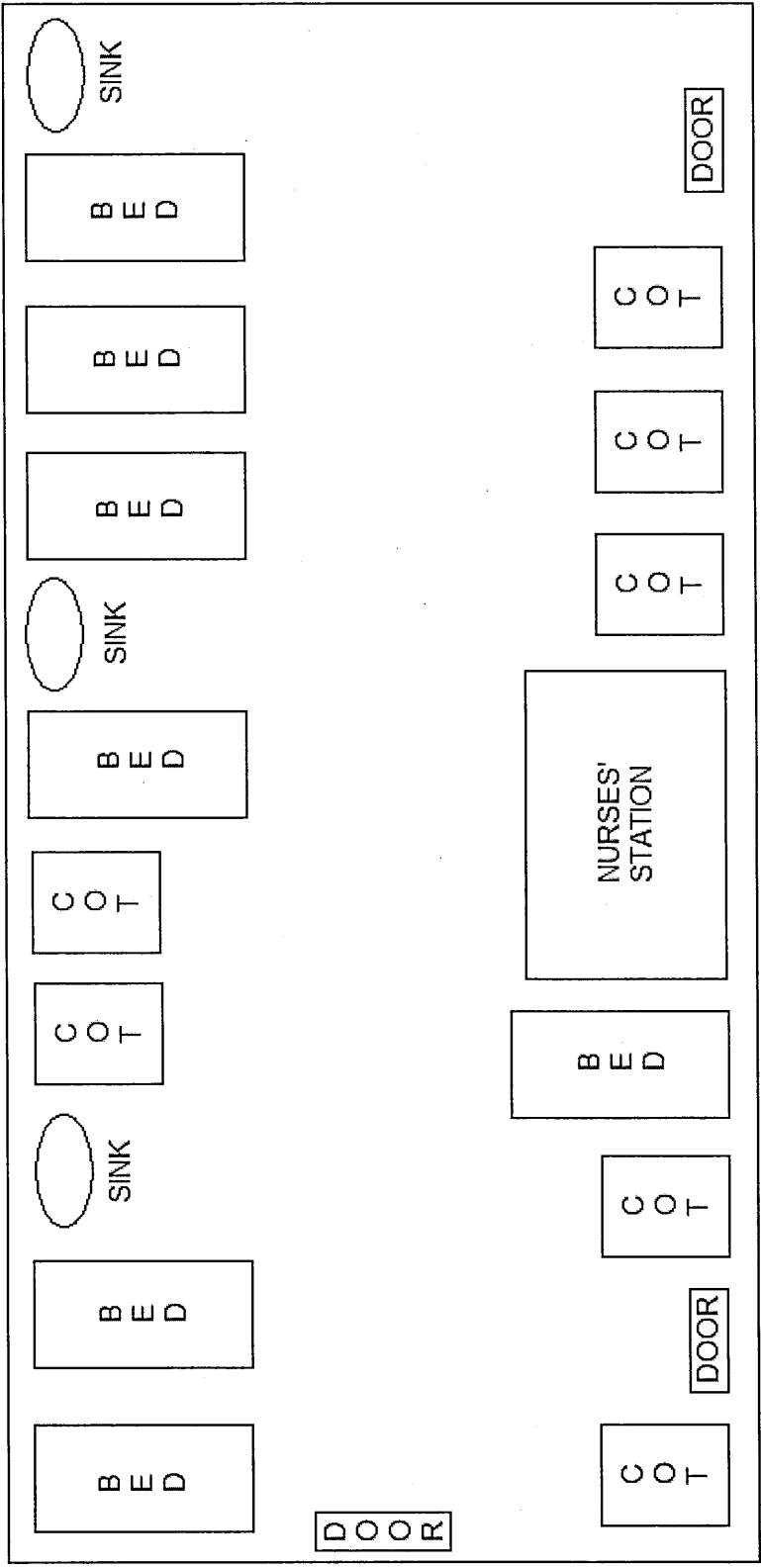
At the time of writing, there are no published studies from Thailand on MRSA carriage or transmission, and data from South and East Asia are limited. Given the significant burden of disease caused by MRSA in Thailand and the high associated mortality as described in Chapters 3-6, it is highly relevant to determine the extent of MRSA colonisation and factors contributing to nosocomial transmission in this setting. The purpose of this study was to establish the prevalence of MRSA carriage in specific hospital settings, and to generate data to inform infection control strategies to reduce MRSA transmission and infection.

Two Intensive Care Unit (ICU) wards at Sappasithiprasong Hospital were chosen for the study. This choice was informed by two observations. First, ICUs in Europe are known to have higher MRSA carriage rates than general wards.^{403,506,507} Second, these wards had harboured patients with MRSA bacteraemia in the previous prospective clinical study (Chapters 4-6). The study methods were as described in Chapter 2.

7.2 Ward details

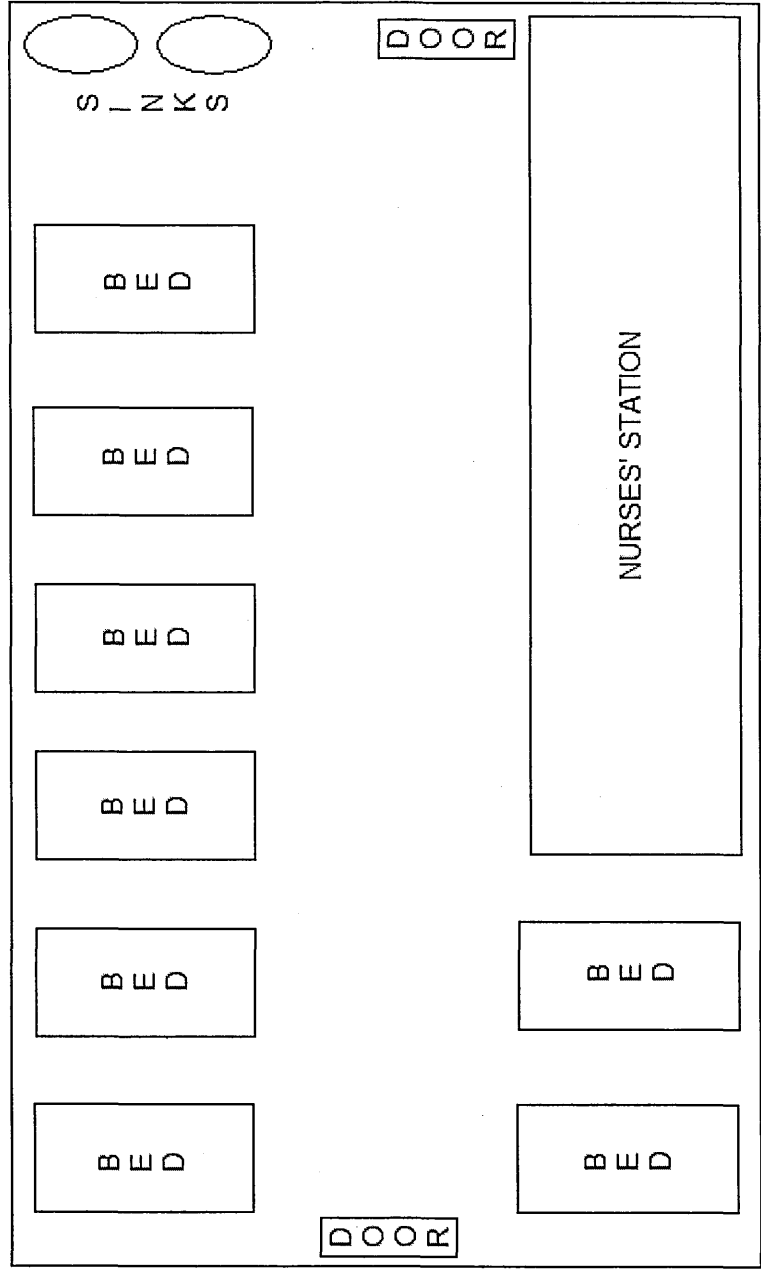
The study wards were a general paediatric ICU, and an adult surgical ICU that provided care to patients with gastroenterological conditions, especially gastrointestinal haemorrhage. The paediatric ICU had 7 beds and 7 cots in open plan (Figure 7.1) and admitted children aged from 1 month to 15 years. The bed occupancy rate during the study period was 91%. The distance between the cots and beds ranged from 47cm to 199cm (median distance 109cm). There were 3 hand washing basins spaced around the ward. The surgical ICU had 8 beds in open plan (Figure 7.2). The bed occupancy rate during the study period was 102%. The distance between beds ranged from 57cm to 72cm (median 63cm). There were 2 hand washing basins at one end of the ward.

Figure 7.1: Diagram showing the layout of the Paediatric Intensive Care Unit



Not drawn to scale

Figure7.2: Diagram showing the layout of the Surgical Intensive Care Unit



Not drawn to scale

There were no isolation facilities on either ICU. Alcohol handrub was available at the end of each bed on both study ICUs. Disposable gloves and masks were readily available but aprons were not used. In the study hospital disposable gloves are collected, sterilised on site and then re-used multiple times until they break. Gloves were used for wound care and procedures involving bodily fluids. The patient to nurse ratio on the study ICUs was 1.3-1.8. Screening for MRSA carriage was not performed on any ward in the study hospital before the start of the study. Patients with MRSA infections cannot be isolated, due to an absence of isolation facilities, but are moved to one end of the row of beds in the ward if possible. These patients are not allocated a specific nurse.

7.3 Infection control

The study hospital has an infection control policy and an infection control team overseen by a doctor and 2 senior nurses. The responsibility for infection control on each ward is allocated to a member of the ward nursing staff who liaises with the 2 senior nurses. Infection control is included in the hospital's teaching programme for the nursing staff. The hospital diagnostic microbiology laboratory informs the senior nurses responsible for infection control if any cultures grow MRSA or extended spectrum beta-lactamase resistant gram-negative pathogens, who then investigate for a potential outbreak.

7.4 Results

7.4.1 Patient characteristics

Surgical ICU: a total of 182 patients were present at the start of the study or admitted over the 3 month study period, of whom 173 patients were enrolled. Nine patients could not be studied because they declined consent (n=6) or they died or were discharged from the study ward before consent could be obtained (n=3). Of the 173 patients enrolled, 1 patient declined any further swabs following the first set.

Paediatric ICU: a total of 126 patients were present at the start of the study or were admitted during the study period, of whom 106 patients were enrolled. Nineteen patients could not be studied because their relatives declined consent (n=8), were

discharged from the study ward before consent could be obtained (n=10) or there was a language barrier for obtaining consent (n=1). In 1 further case the patient's guardian gave consent but the child refused to be swabbed and was then discharged from the study ward; this case was excluded from any analysis.

The patient characteristics for each ward are given in Table 7.1. In keeping with the admission policies of the study wards, the patients on the surgical ICU were aged 15 to 93 years (median 62 years, interquartile range (IQR) 49-73 years) and those on the paediatric ward were aged 1 month to 15 years (median 2 years, IQR 8 months – 10 years). There was a significant predominance of male patients on both the surgical and paediatric ICU wards (68% $p<0.001$ and 60% $p=0.04$, respectively). The most common diagnoses for the paediatric patients were: respiratory failure (40%, n=42), sepsis (20%, n=21) and seizures (13%, n=14). On the surgical ward patients were commonly admitted with gastrointestinal haemorrhage (43%, n=75); other reasons for admission included: post-operative care following abdominal or retroperitoneal surgery (24%, n=42), septic shock (12%, n=20) and peritonitis (6%, n=11).

The pattern of admissions to the study ICU wards was quite different between the paediatric and adult wards, as detailed in Table 7.1. The turnover of patients was significantly more rapid on the surgical ICU (median length of stay for the first admission 2 days (IQR 1-4) versus 3 days (IQR 2-7), $p=0.009$). For those patients already admitted to the study ward prior to the onset of study enrolment, the length of time on the study ward before enrolment was significantly longer for the paediatric patients than the surgical patients (median length 31 days (IQR 6-76) versus 3 days (IQR 1-10), $p=0.02$), which was in keeping with the longer lengths of stay on the paediatric ward. The difference in the numbers of patients already on the study ward at the start of enrolment between the 2 ICUs merely reflects the discrepancy in the number of beds on each unit. Paediatric patients also had significantly more repeat admissions to the study ICU than the adult surgical patients ($p<0.001$).

Table 7.1: Patient characteristics for the 2 Intensive Care Unit (ICU) study wards

		Surgical ICU ward (n=173)	Paediatric ICU ward (n=106)
Age	<i>median (IQR)</i>	62 years (49-73 years)	2 years (8 months-10 years)
Sex (male)	<i>number (% of ward total)</i>	118 (68%)	64 (60%)
1st Admission to study ICU			
On ICU ward at start of study	<i>number (% of ward total)</i>	7 (4%)	13 (12%)
- Length of inpatient stay before study	<i>median (IQR)</i>	3 days (1-10 days)	31 days (6-76 days)
Inpatient at study hospital prior to ICU	<i>number (% of ward total)</i>	87 (50%)	49 (46%)
- Length of inpatient stay before ICU	<i>median (IQR)</i>	1 day (0-2 days)	1 day (0-8 days)
Direct admission to study ICU	<i>number (% of ward total)</i>	79 (46%)	44 (42%)
- Admitted from another health centre		70/79 (89%)	36/44 (82%)
Length of stay during 3 month study period	<i>median (IQR)</i>	2 days (1-4 days)	3 days (2-7 days)
2nd Admission to study ICU			
Number of patients with 2 nd admission	<i>number (% of ward total)</i>	4 (2%)	15 (14%)
Length of hospital admission between 1 st and 2 nd ICU admission	<i>range; ^{*1} median (IQR)</i>	0-4 days	8 days ^{*2} (6-15 days)
Length of stay during 3 month study period	<i>range; ^{*1} median (IQR)</i>	1-12 days	2 days (1-22 days)
3rd Admission to study ICU			
Number of patients with 3 rd admission	<i>number (% of ward total)</i>	0	3 (3%)
Length of hospital admission between 2 nd and 3 rd ICU admission	<i>range</i>		0-42 days ^{*3}
Length of stay during 3 month study period	<i>range</i>		3-25 days
4th Admission to study ICU			
Number of patients with 4 th admission	<i>number (% of ward total)</i>	0	1 (1%)
Length of hospital admission between 3 rd and 4 th ICU admission			1 day
Length of stay during 3 month study period			14 days

^{*1} First column (i.e. Surgical ICU) shows range because there are only 4 data points and second column (i.e. Paediatric ICU) shows median (IQR).

^{*2} 2 patients discharged home for 2 and 15 days respectively in the interval between their 1st and 2nd ICU admissions

^{*3} 1 patient discharged home for 36 days in the interval between their 2nd and 3rd ICU admission

7.4.2 Patient carriage and transmission

On the adult surgical ICU ward, 75 of the 173 patients (43%) were positive for *S. aureus* on at least one screening swab, 27 of which were MRSA (16% of patients admitted to the unit during the study period). On the paediatric ICU, 63 of 106 patients (59%) were positive for *S. aureus* on at least one screening swab, 33 of which were MRSA (31% of patients admitted to the unit during the study period). The difference in MRSA carriage rates between the two units was highly significant ($p=0.003$).

A detailed breakdown of the results of the differing swabs and other samples is shown in Table 7.2. Initially taking the results from the whole patient cohort, on both the adult surgical ICU and the paediatric ICU tracheal suction samples detected the highest proportion of MRSA carriers. Predictably, wound swabs were taken significantly more frequently on the surgical ICU, with at least one wound swab taken from 51% versus 11% of all patients, respectively ($p<0.001$). Urine samples were taken significantly less frequently on the paediatric ICU, 8% versus 93% of all patients, respectively ($p<0.001$), as young children wore nappies rather than being catheterised. Amongst patients carrying MRSA, nasal swabs were the most common swab type to be positive, followed by throat swabs and tracheal samples with 2nd and 3rd place differing between the 2 wards. Urine samples had the lowest rate of positivity for MRSA amongst both the adult and paediatric patients.

Amongst the patients carrying MRSA, the paediatric patients had more screens performed compared to the adult surgical patients, 3 screens (IQR 2-4) versus 4 (IQR 3-9), respectively ($p=0.05$) and a significantly greater number of positive screens, 1 screen (IQR 1-3) versus 3 (IQR 1-7), respectively ($p=0.04$). There was a trend for the paediatric patients to have a higher proportion of patients with more than 50% of screens positive for MRSA compared with adult surgical patients, 73% versus 48% respectively ($p=0.065$). These results are likely to reflect the longer lengths of stay and higher number of re-admissions amongst the paediatric patients.

Table 7.2: Details of swabs and other samples

	Surgical ICU All patients (n=173)		Paediatric ICU All patients (n=106)	
	Patients with swabs taken during study	Patients with swabs that grew MRSA	Patients with swabs taken during study	Patients with swabs that grew MRSA
Axillary swab	173 (100%)	6 (3%)	106 (100%)	15 (14%)
Nasal swab	172 (99%)* ¹	18 (10%)	106 (100%)	30 (28%)
Throat swab	137 (79%)	14 (10%)	94 (89%)	12 (13%)
Tracheal suction sample	66 (38%)	9 (14%)	30 (28%)	15 (50%)
Urine sample (catheterised)	161 (93%)	3 (2%)	8 (8%)	1 (13%)
Wound swab	88 (51%)	9 (10%)	12 (11%)	4 (25%)
	Surgical ICU MRSA carriers (n=27)		Paediatric ICU MRSA carriers (n=33)	
	Carriers with swabs taken during study	Carriers with swabs that grew MRSA	Carriers with swabs taken during study	Carriers with swabs that grew MRSA
Axillary swab	27 (100%)	6 (22%)	33 (100%)	15 (45%)
Nasal swab	27 (100%)	18 (67%)	33 (100%)	30 (91%)
Throat swab	22 (81%)	14 (64%)	24 (%)	12 (50%)
Tracheal suction sample	17 (63%)	9 (53%)	21 (%)	15 (71%)
Urine sample (catheterised)	27 (100%)	3 (11%)	4 (%)	1 (25%)
Wound swab	18 (67%)	9 (50%)	10 (%)	4 (40%)

All data are number (%).

*¹ One patient had tubing in both nostrils throughout their admission so it was not possible to obtain a nasal swab sample.

The characteristics of MRSA carriage and acquisition are shown in Table 7.3. On the surgical ICU over half the patients (56%) acquired MRSA whilst they were admitted to the study ward. A third of the patients arrived on the ward for the first time during the study as MRSA carriers. Over half (52%) the patients only had one screen positive for MRSA and the majority of these involved 1 swab that grew MRSA. By comparison, on the paediatric ICU equal numbers of patients acquired MRSA whilst they were admitted to the study ward as arrived on the ward for the first time during the study as MRSA carriers. More than half (58%) the paediatric patients carried MRSA continuously, once acquired, and 2 of the 3 patients who intermittently carried MRSA had 24 or more positive screens. The proportion of patients who acquired MRSA on the study ward during the study period was not significantly different between the 2 ICUs ($p=0.19$) nor was the pattern of MRSA carriage ($p=0.36$). The median time to MRSA acquisition from admission to the study hospital was significantly shorter on the surgical ICU, 4 days (IQR 3-6 days) versus 11 days (IQR 2-17 days) ($p=0.013$). However, the median time to MRSA acquisition from admission to the study ICU was not significantly different between the surgical and paediatric ICUs, 3 days (IQR 1-4 days) and 2 days (IQR 1-9 days) respectively, ($p=0.76$). On both study ICUs the majority of patients had received antibiotic therapy prior to the detection of their MRSA. Of the 9 patients (4 surgical, 5 paediatric) who "cleared" their MRSA 6 (2 surgical, 4 paediatric) had only one screen negative for MRSA prior to discharge from the study ICU so some of these cases may in fact reflect intermittent carriage.

Table 7.3: Characteristics of MRSA carriage and acquisition

Characteristic	Surgical ICU MRSA cases (n=27)	Paediatric ICU MRSA cases (n=33)
MRSA acquisition		
MRSA first detected from:		
- 1 st screen	11 (41%)	17 (52%)
- 2 nd screen	12 (44%)	8 (24%) ²
- 3 rd screen	2 (7%) ¹	4 (12%) ³
- 4 th screen	1 (4%)	2 (6%)
- 5 th screen	1 (4%)	1 (3%)
- 10 th screen	-	1 (3%)
MRSA acquired		
- Before start of study if on ward at start of study (1 st screen of study detected MRSA)	2 (7%)	5 (15%)
- Before arrival on study ward (1 st screen detected MRSA)	9 (33%)	12 (36%)
- On study ICU ward during study period	15 (56%)	12 (36%)
- On another ward in hospital (1 st screen of re-admission to study ward detected MRSA)	1 (4%)	4 (12%)
On ward at start of study	(n=2)	(n=11) <i>median (IQR)</i>
- Days in study hospital prior to first MRSA detected	15 and 22 days	47 (17-90) days
- Days on study ICU prior to first MRSA detected	1 and 5 days	4 (0-9) days
Admission during study	(n=25) <i>median (IQR)</i>	(n=22) <i>median (IQR)</i>
- Days in study hospital prior to first MRSA detected	4 (3-6) days	11 (2-17) days
- Days on study ICU prior to first MRSA detected (adjusted for time between study ICU admissions)	3 (1-4) days	2 (1-9) days
Antibiotic therapy received prior to first MRSA screen	24 (89%)	29 (88%)
MRSA carriage		
One positive screen only	14 (52%)	11 (33%)
- 1 swab from screen grew MRSA	10	9
- 2 swabs from screen grew MRSA	4	2
Intermittent carriage (all swabs of one screen negative between two positive screens)	2 (7%)	3 (9%)
Continuous carriage (at least one swab in every screen positive)	11 (41%)	19 (58%)
"Clears" MRSA (all swabs of screen negative prior to discharge from study ward)	4 (14%)	5 (15%)

Data are number (%), unless otherwise specified

*¹ In 1 of these cases it was the 1st screen after re-admission to the study ICU

*² In 2 of these cases it was the 1st screen after re-admission to the study ICU

*³ In 2 of these cases it was the 1st screen after re-admission to the study ICU

7.4.3 Isolate antibiogram profiles and sequence types

The majority of MRSA isolates were multi-resistant *in vitro* to the panel of antibiotics tested as shown in Table 7.4. Combined resistance to ciprofloxacin, clindamycin, erythromycin, gentamicin, netilmicin, tetracycline and trimethoprim-sulphamethoxazole was seen in 94% of isolates. The first MRSA isolate obtained for each patient, with preference given to nasal samples, underwent molecular analysis to determine the sequence type. Of these initial MRSA isolates 97% (58/60) were sequence type 239 (ST 239). In those 5 patients who carried MRSA intermittently, the first MRSA swab after a screen of all negative swabs was also genotyped and all were ST 239. By sequence type these subsequent isolates were unchanged from the original isolate for that patient, however by antibiogram the isolate had changed in 4 out of 5 patients (Table 7.5), although only by 1 antibiotic in each case.

Table 7.4: Antibiotic resistance rates from *in vitro* disk diffusion testing

Antibiotic	Resistance rate
Cefoxitin	100%
Chloramphenicol	2%
Ciprofloxacin	99%
Clindamycin	98%
Erythromycin	100%
Fusidic acid	23%
Gentamicin	99%
Mupirocin	33%
Netilmicin	98%
Penicillin	100%
Rifampicin	21%
Tetracycline	99%
Trimethoprim-sulphamethoxazole	99%
Vancomycin	0%

Table 7.5: Antibigram and sequence type data for the initial MRSA isolates and the isolates recovered after a negative screen in the intermittent MRSA carriers

Patient	Sample	Sequence type (by MLST)	Changes in antibiogram	
			Fusidic acid	Rifampicin
Intermittent carrier 1	Initial MRSA isolate	239	-	-
	Isolate recovered after 1st negative screen	239	-	-
	Isolate recovered after 2nd negative screen	239	-	-
Intermittent carrier 2	Initial MRSA isolate	239	Susceptible	-
	Isolate recovered after 1st negative screen	239	Resistant	-
Intermittent carrier 3	Initial MRSA isolate	239	-	Resistant
	Isolate recovered after 1st negative screen	239	-	Susceptible
Intermittent carrier 4	Initial MRSA isolate	239	Resistant	-
	Isolate recovered after 1st negative screen	239	Susceptible	-
Intermittent carrier 5	Initial MRSA isolate	239	-	Susceptible
	Isolate recovered after 1st negative screen	239	-	Resistant

7.4.4 MRSA infection

Amongst the 33 patients on the paediatric ICU who carried MRSA, 2 developed MRSA bacteraemia (6%). In each case their MRSA carriage was detected prior to the isolation from blood cultures. Although the isolates from bacteraemia were not obtained for further re-testing their antibiograms from the hospital diagnostic microbiology laboratory matched those of the carriage strains. Whilst there was no MRSA detected from blood cultures on the surgical ICU, 53 patients (31%) had temperatures recorded $\geq 38^{\circ}\text{C}$ from whom no cultures were taken.

7.4.5 Nursing staff

Covert hand washing surveys on each of the wards showed that compliance was 0-17% (mean 9%) on the surgical ICU and 0-14% (mean 8%) on the paediatric ICU. On the surgical ICU, 22 nursing staff were screened and 1 nurse was found to carry MRSA on a nasal swab on a single screen. The nurse was not screened again because by chance she was not on duty the next 2 times screening was undertaken. From the 15 nursing staff screened on the paediatric ICU, 4 were colonised with MRSA; 1 from finger imprints and 3 from nasal swabs. The nurse with the hand carriage was not a nasal carrier on a single screen and again by chance was not on duty the next 2 times screening was undertaken. Amongst the 3 nurses with nasal carriage, one was a persistent nasal carrier with positive nasal swabs from all 3 screenings; the others were not screened after their MRSA positive swab but one of these 2 had been screened previously and had not grown MRSA. There was a significant association between length of time working and carriage status: the nurses colonised with MRSA had worked for a shorter length of time than those not carrying MRSA, 7 years (IQR 4-8 years) compared with 12 years (IQR 8-19 years), $p=0.04$. There was a trend which did not reach significance between MRSA colonisation and the seniority of the different nursing grades in Thailand ($p=0.07$); all the MRSA carriers were registered nurses, who are more senior than technical nurses which is the grade above nurse aide. All of the nurses worked full time.

7.5 Discussion

This study of MRSA carriage has clearly demonstrated that acquisition and transmission of MRSA within the study ICUs was occurring at a considerable rate. However, MRSA carriage and transmission did not appear to be solely an issue on the ICUs; 5 patients re-admitting to study ICUs were found to be MRSA carriers for the first time. It was not possible from the study design to determine how many patients who were on the ward prior to the start of the study or who were admitted to other wards in the hospital before the study ICUs, had acquired MRSA on other hospital wards. The rate of MRSA carriage was significantly higher on the paediatric ICU, where the patients' length of hospital and ICU stays and number of ICU admissions were significantly higher. Increasing length of hospital stay as a risk factor for the development of MRSA carriage has been established in developed countries^{401,403,405} and the findings of this study would support this as a risk factor in a developing country setting. The median time to acquisition of MRSA after admission to the study ICU was rapid on both the paediatric and surgical ICUs, 2 and 3 days respectively. This meant that even though the turnover of patients on the surgical ICU was high (median length of stay 1 day), there was a considerable rate of MRSA carriage. There was a high input pressure of patients arriving on the ward already carrying MRSA, which would facilitate the rapid dissemination amongst the other ICU patients. Additionally, the antibiotic pressure was high with almost 90% of patients receiving antibiotic therapy before their MRSA carriage was detected.

The multi-resistant nature of the *in vitro* antibiotic resistance profiles of the MRSA carriage isolates and the fact that they belonged to a single clone of ST 239 supported the notion that these were healthcare-associated. ST 239 is known to be the dominant MRSA clone causing hospital-acquired infection in Thailand²²⁴ and is the predominant clone across South and East Asia.⁵⁰⁸ Although CA-MRSA is a considerable problem in many countries and more recently has begun to contribute to MRSA transmission within healthcare facilities,⁵⁰⁹⁻⁵¹² this was not a feature of this study. The similarity of the carriage isolates was a limiting factor of the study because establishing the epidemiology of transmission in more detail from the current data was not possible. A typing technique,

such as pulsed field gel electrophoresis, might provide some insights into variability within the ST 239 clone which could be used to study transmission dynamics in greater detail.

Since hand washing compliance rates were poor on both ICUs, MRSA transmission from patient-to-patient via healthcare worker hands was likely to have been a major contributor to the high carriage rates observed. The finding that nurses who were MRSA carriers had a significantly shorter length of time spent working as a nurse could reflect the duties of nurses of differing experience. For example, newer nurses may have to perform more duties involving bodily fluids, which is a task known to be associated with a higher rate of contamination. Lack of experience could correlate with lower hand washing compliance, although all the MRSA carriers were registered nurses and in Thailand registered nurses receive the longest training of the 3 nursing grades.

The lack of interest in and concern with MRSA carriage and transmission as developing country problems, as demonstrated by the scarcity of published literature, seems unjustified in light of this study. Rates of MRSA carriage up to 31% demand urgent attention. The poor hand washing compliance indicates that hand washing is an essential aspect of infection control to target and could be achieved largely without additional expense. Further measures, such as increasing the space between beds, isolation rooms for patients with MRSA, increasing the number of hand wash basins on the wards or disposing of and not re-sterilising gloves require considerable additional investment in resources. Studies to specifically assess the cost-effectiveness of such expensive interventions would be needed prior to implementation. Addressing MRSA carriage is of major importance because patients are most likely to develop an infection with their own carriage strain,^{54,56} so patients carrying MRSA are at a higher risk of MRSA infection, from which the mortality is often higher than MSSA.⁵¹³⁻⁵¹⁵ There were 2 patients on the paediatric ICU who developed MRSA bacteraemia following MRSA carriage, representing 6% of all the MRSA carriers on that ward over a short time period of this study (3 months).

The World Health Organisation is promoting hand hygiene as their first global patient safety challenge, under the campaign "Clean care is safer care".⁴⁷⁴ Although Thailand has pledged its commitment to addressing healthcare-associated infections, a

number of other countries in South and East Asia, including neighbours of Thailand, have not yet signed up to this WHO initiative. Drug-resistant bacteria are not constrained by country borders and so tackling healthcare-associated infections, including MRSA, requires a concerted international effort in order to succeed.

Chapter 8. Concluding comments

For many clinicians and researchers around the world, *S. aureus* is not a pathogen of particular relevance to the tropics. A review of the literature on *S. aureus* in resource-restricted countries in South and East Asia to assess this assumption threw up more questions than answers. A major question was whether the lack of published studies was due to a low prevalence of *S. aureus* disease in this region, or more likely based on anecdotal experience in resource-restricted Asia, that lack of resources including diagnostic microbiology facilities led to under-representation in the literature. This body of work convincingly demonstrates that in provincial Thailand at least, *S. aureus* is responsible for substantial morbidity and mortality. It is improbable that this is an isolated finding because the published literature includes *S. aureus* as one of the causative organisms for a diverse range of clinical manifestations from countries across the Asian region. Further work is needed from more resource-restricted countries in South and East Asia to determine the full extent of the disease burden due to *S. aureus*. Hopefully the publication of this body of work will encourage other researchers to consider this as a topic for future study.

A further consideration is whether *S. aureus* behaves differently in a tropical climate, perhaps causing less severe disease, a factor that could contribute towards the lack of published data. However, the work contained in this thesis establishes that with regard to epidemiology, risk factors for disease and clinical presentations, *S. aureus* in the tropical developing country studied mirrors that of temperate developed countries. *S. aureus* commonly caused invasive disease and severe sepsis amongst those with bacteraemia which refutes the suggestion of a less virulent pathogen. The finding that the mortality rate for *S. aureus* bacteraemia was much higher in provincial Thailand than in developed countries again does not support a hypothesis that *S. aureus* is less virulent in this setting.

Mortality from sepsis is a global problem but disproportionately affects developing countries. Whilst major steps in improving supportive sepsis management have been

made in developed countries, in large part due to the 'Surviving Sepsis Campaign' guidelines, outcomes in developing countries remain very poor. The findings from this work that many of the essential elements, namely appropriate sampling for diagnostic microbiology, adequate fluid resuscitation, prompt antibiotic therapy and procedures for infectious source control, are within the capability of resource-restricted health centres are very encouraging. Work on adapting these sepsis guidelines and implementing them in a broad variety of resource-restricted health settings in developing countries across the world is urgently needed. Research funding bodies placing their support behind such a strategy would be invaluable.

The acquisition of antibiotic resistance by important pathogens is a high profile concern in developed countries. This work describing the considerable rates of MRSA in bacteraemia and the higher mortality rates attributable to MRSA indicates that this issue is just as relevant in a resource-restricted setting. Given the challenges faced by developed countries in tackling drug resistance even when access to antibiotic is regulated, meaningful progress on drug resistance in developing countries is unlikely to be achieved unless there is political will to control and regulate antibiotic availability and to address the manufacturing and distribution of sub-standard antibiotic drugs.

The debate over whether Panton-Valentine Leukocidin (PVL) is a virulence determinant and its potential mode of action remains controversial and hotly contested. This work adds importantly to the evidence because it describes results in an unselected cohort of patients, which has been distinctly lacking in the literature to date. The finding that PVL gene-positive isolates were associated with survival was unexpected and challenges the current epidemiological support for its role in virulence.

The work on MRSA carriage and transmission within 2 Intensive Care Units highlights that MRSA carriage is an endemic problem of considerable magnitude. Urgent infection control interventions are clearly required. The most basic and inexpensive infection control measure is hand washing and this study revealed that there was much room for improvement on compliance rates. An obvious follow on study would be to

determine the effectiveness of a hand washing campaign on MRSA carriage rates and infections.

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Appendix 1. Retrospective *Staphylococcus aureus* Study Form

Study number _____

Ubon Ratchathani hospital number _____

Patient Information

Last name _____ First name _____

Date of Birth ____/____/____ Age ____

Gender: Male Female

Occupation: _____

Hospital Admission Information

Admit Date ____/____/____

Discharge Date ____/____/____

Community vs hospital-acquired infection

Positive blood culture taken within 48 hours of admission Yes No

Healthcare contact in last year? Yes No

Details of attendance (use code) _____

Source Information

Initial source of infection (give code) _____

Was surgery performed within previous 30 days of infection at the site of this surgery?
Yes No

If yes, what type of surgery was performed? _____

If yes, when was the surgery performed? ____/____/____

Prosthesis present at time of infection Yes No

Give code of type of prosthesis _____

Removable source of bacteraemia present Yes No

Was a removable focus removed? Yes No

Number of days from 1st blood culture positive for *S. aureus* until removal of removable source _____ days

Past Medical History

Chronic illness (give all relevant codes please) _____

Is the patient allergic to penicillin? Yes No

Blood Culture Information

Date of first positive blood culture for *S. aureus* ___/___/___

White cell count at time of 1st positive blood culture _____ x 10⁹/L

Number of blood cultures drawn (starting with 1st blood culture positive for *S. aureus*):___

Number of blood cultures positive (starting with 1st blood culture positive for *S. aureus*):___

Do the blood cultures meet Duke major criteria? Yes No

Do the blood cultures meet Duke minor criteria? Yes No

Blood Cultures

Date	# drawn	# Positive	MSSA/MRSA
/ /			
/ /			
/ /			
/ /			
/ /			
/ /			
/ /			

Temperature >38°C continued to be recorded >72 hours after 1st blood culture positive for *S. aureus*? Yes No

No. of days after 1st blood culture positive for *S. aureus* before effective antibiotic therapy started? _____ days

Complications

Metastatic infection is defined as a site of S. aureus infection resulting from spread from an initial site (via bloodstream seeding or direct extension).

Metastatic infection Yes No

Give infection code and for each infection give code for confirmation:_____

Echocardiogram performed? Yes No

Date Echocardiogram performed ___/___/___

Echo result (give codes) _____

Duke Major echocardiographic criteria met? Yes No

Duke Minor echocardiographic criteria met? Yes No

Heart Valve Information

Has the patient had a previous episode of infective endocarditis (IE)? Yes No

Does the patient have a known cardiac condition that predisposes to endocarditis?

Yes No If yes, code of disease: _____

Does the patient have a prosthetic valve present? Yes No

Which valve(s) (give codes)? _____

Which type of valve (give code)? _____

Treatment

Was the patient receiving antibiotic therapy at time blood culture taken? Yes No

Which antibiotics? _____

Which antibiotics were used after 1st blood culture positive for *S. aureus*? _____

How many days in total of antibiotic therapy were received? _____ days

How many days of effective antibiotic therapy were received? _____ days

Outcome

Give outcome code: _____

If the outcome was death, when did the patient die? ____/____/____

Codes for form

Healthcare contact codes

1. In-patient stay
2. Out-patient appt
3. Attendance for regular interventions e.g. haemodialysis, blood transfusions, radiotherapy

Initial source of bacteraemia (choose one):

- | | | |
|-------------------------|------------------------------------|-----------------------------|
| 1 Abscess | 9 Gangrene | 17 Empyema |
| 2 Cellulitis | 10 Septic arthritis | 18 Mediastinitis |
| 3 Skin ulcer | 11 Infected joint prosthesis | 19 Sinusitis |
| 4 Burn | 12 Peripheral intravenous catheter | 20 Percutaneous nephrostomy |
| 5 Wound – trauma | 13 Central intravenous catheter | 21 Peritoneal dialysis |
| 6 Wound – surgical | 14 Vascular graft | 22 Biliary tract |
| 7 Dermatitis/ psoriasis | 15 Primary AV fistula | 23 None/ unknown |
| 8 Furuncle | 16 Pneumonia | 24 Other |

If "other" was chosen above, list the source: _____

Prosthesis Type

1. Short-term peripheral intravenous catheter/cannula
2. Short-term central intravenous catheter/cannula
3. Indwelling peripheral intravenous catheter/cannula e.g. PICC line
4. Indwelling central intravenous catheter/cannula e.g. Hickman line
5. Intravascular graft
6. Haemodialysis graft
7. Prosthetic heart valve
8. Pacemaker/ defibrillator
9. Joint replacement
10. Orthopaedic rod/ plate/ screws

Chronic illness codes

1. Severe lung disease e.g. chronic obstructive pulmonary disease, emphysema, tuberculosis
2. Severe cardiac disease e.g. rheumatic heart disease, ischaemic heart disease
3. Severe liver disease e.g. chronic hepatitis C, auto-immune liver disease
4. Chronic renal failure
5. Dialysis-dependent
6. Diabetes mellitus
7. Lymphoma or leukaemia
8. Received radiotherapy or chemotherapy
9. Currently on immunosuppressive therapy, including high dose steroids
10. Recipient of an organ transplant
11. Immunosuppressed e.g. HIV infection

Metastatic infections

- A. Endocarditis
- B. Lung emboli
- C. Brain emboli
- D. Septic arthritis
- E. Osteomyelitis
- F. Glomerulonephritis

Confirmed by

1. Physical examination
2. Culture
3. Radiology

4. Echocardiography
5. Other

Echo result

Valve(s) involved:

- A. Mitral valve
- B. Aortic valve
- C. Tricuspid valve
- D. Pulmonary valve

Defects seen:

1. More than mild regurgitation
2. Valve prolapse
3. Thickened valve leaflet
4. Prosthetic valve
5. Vegetation
6. Calcified valve
7. Perforated valve
8. Paravalvular abscess
9. Ruptured chordae

Predisposing heart conditions for endocarditis

1. Rheumatic heart disease
2. Congenital heart disease

Place of prosthetic valve

- A. Mitral valve
- B. Aortic valve
- C. Tricuspid valve
- D. Pulmonary valve

Type of prosthetic valve

1. Metallic valve
2. Tissue valve
3. Ring/ support device

Outcome

1. Cure
2. Complications of *S. aureus* (haematogenous seeding)
3. Death due to *S. aureus*
4. Death due to other causes

Prospective study of invasive *Staphylococcus aureus* disease – Data collection sheet

Please fill in the blanks or circle the appropriate choices.

Study number _____ Date of 1st visit ____/____/____
Ubon Ratchathani hospital number _____

Patient Information

First name _____ Last name _____ ID card number _____

Date of Birth ____/____/____ Age _____

Gender: Male Female

Occupation: _____

Phone numbers: home _____ mobile _____

Address: _____

Hospital Information

Admit Date ____/____/____ Time _____
Discharge Date ____/____/____ Time _____ Place _____

Ward _____ Consultant _____

Details on admission/ when culture taken

Date 1st positive culture for *S. aureus* taken ____/____/____

On that date:

Maximum temperature _____ °C

Did the patient have a significant BP abnormality (i.e. systolic ≤90, diastolic ≤60)?

Yes No N/A

Maximum pulse _____ bpm

Maximum respiratory rate _____ bpm

White cell count at time 1st positive culture taken _____ x 10⁹/L
date ____/____/____

Procedure

Was a procedure used to treat the staphylococcal infection? Yes No

Date ____/____/____

	Surgical debridement		Abscess drainage (I+D)
	Surgical removal of graft/ prosthesis		Joint wash out (arthrocentesis)
	Other,		

Investigations

Investigations performed to look for presence of pus collections? Yes No

Date	Investigation	Result

Treatment

When was antibiotic treatment started? / /
When was antibiotic treatment finished? / /

Empirical antibiotic therapy: Ideal Staph cover Covers Staph but not ideal Does not cover Staph
Date effective antibiotic therapy started / /

Antibiotic regimen:

Antibiotic given	Start date	Stop date	Route	Dose and frequency	No. of days

How many days in total were antibiotics used?

Reason vancomycin therapy used:

	MRSA
	Suspected MRSA
	Penicillin Allergy
	Inappropriate (None of above reasons apply)

How many days elapsed from starting effective intravenous antibiotic therapy until the day when maximum temperature <38°C?

On what date did the patient defervesce*? / / Was it within 72 hours?
Yes No N/A

*(Temperature consistently <38 °C and remains <38 °C.)

Symptom Information

Number of days before culture taken that the patient first had any symptoms or signs? ____
admission _____

History:

Fever	Yes	No	Infected skin lesion	Yes	No
Chills and Sweats	Yes	No	Chest pain	Yes	No
Malaise	Yes	No	Cough with sputum	Yes	No
CNS involvement	Yes	No	Abdominal pain	Yes	No
Haematuria	Yes	No	Joint pain	Yes	No
Skin changes (e.g. petechiae)	Yes	No	Muscle pain	Yes	No
Shortness of breath	Yes	No	Neonates – poor feeding	Yes	No
Peripheral oedema	Yes	No	Neonates – maternal mastitis	Yes	No

Past Medical History

Significant lung disease e.g. COPD	Yes	No	Recipient of organ transplant	Yes	No
Significant cardiac disease e.g. IHD, rheumatic disease	Yes	No	Currently on immuno-suppressive therapy, inc high dose steroids ($\geq 30\text{mg/day}$)	Yes	No
Significant liver disease e.g. Hep C	Yes	No	Lymphoma or leukaemia	Yes	No
Chronic renal failure (creatinine $>200\mu\text{mol/L}$; $>2\text{mg/dL}$)	Yes	No	Other cancer	Yes	No
Dialysis-dependent	Yes	No	Received radiotherapy or chemotherapy	Yes	No
Diabetes mellitus	Yes	No	HIV infection	Yes	No
Rheumatoid arthritis/ osteoarthritis	Yes	No	On HAART	Yes	No

Other significant condition not mentioned above? _____

Is the patient allergic to penicillin? Yes (details: _____) No Not known

Heart Valve information:

Has the patient had a previous episode of infective endocarditis (IE)? Yes No

Does the patient have a known cardiac condition that predisposes to endocarditis?
Yes No (Rheumatic heart disease/ congenital heart disease)

Does the patient have a replacement valve present? Yes No

Valve(s): Mitral Aortic Tricuspid Pulmonary
Type of valve: Metallic valve Tissue valve Ring/ support device

MRSA risk factors

Healthcare contact in last year? Yes No

In-patient stay

Hospital type: Local/ District Ubon Other e.g. BKK _____

Dates of most recent stay _____

☐ 2-3 in-patient stays in last year

☐ >3 in-patient stays in last year

Out-patient visits

Clinic/ intervention: OPD Dialysis Chemotherapy Radiology Other _____

Frequency: weekly every 1-2 months/ 3-4 months/ 5-6 months/ >6 months

As a relative

OPD/ single visit Stayed overnight Multiple visits to ward

Other MRSA risk factors¹

Resident of care/nursing home	Yes	No	IVDU (current or ever)	Yes	No
Healthcare worker	Yes	No	Household contact of IVDU	Yes	No
Household contact of healthcare exposure	Yes	No	Prison in last year	Yes	No
Close contact of similar infection	Yes	No	Household contact of prison exposure	Yes	No
Antibiotics taken in last month	Yes	No	Living on streets in last year	Yes	No
Antibiotics taken for this infection	Yes	No	Competitive contact sport in last month	Yes	No
Presence of wounds/ skin breaches	Yes	No			

¹Moran GJ et al NEJM 355(7): 666-674

Physical Examination at time of 1st positive *S. aureus* culture result

Patient continues to have a temperature $\geq 38^{\circ}\text{C}$? Yes No Maximum temp _____ $^{\circ}\text{C}$

Examination findings

Skin	Normal	Yes	No	Pustule/abscess	Yes	No
	Petechiae	Yes	No	Cellulitis	Yes	No
	Macules/ Janeway lesions	Yes	No	Ulcer	Yes	No
	Osler's nodes	Yes	No	Infected cannula site	Yes	No
	Vasculitis lesions/infarcts	Yes	No	Infected wound site	Yes	No
	Ecchymosis	Yes	No	Infected graft site	Yes	No
	Other	Yes	No			
Nails	<i>Normal</i>	Yes	No	<i>Infected</i>	Yes	No
	<i>Splinter haemorrhages</i>	Yes	No			
	<i>Other</i>	Yes	No			
Murmur	None	Yes	No	<i>New Murmur</i>	Yes	No
	Systolic	Yes	No	<i>Signs of heart failure</i>	Yes	No
	Diastolic	Yes	No			
	Other	Yes	No			
Resp	<i>Normal</i>	Yes	No	Signs of effusion/ empyema	Yes	No
	<i>Signs of pneumonia</i>	Yes	No	<i>CXR confirms examination</i>	Yes	No
	<i>Other</i>	Yes	No			
GI	Normal	Yes	No	<i>Hepatomegaly</i>	Yes	No
	Splenomegaly	Yes	No	Tenderness	Yes	No
	<i>Other</i>	Yes	No			
Neuro	<i>Normal</i>	Yes	No	<i>Visual field defect</i>	Yes	No
	<i>Focal neurological signs in limbs</i>	Yes	No	<i>Meningitis signs</i>	Yes	No
	<i>Confusion/ reduced GCS</i>	Yes	No			
	<i>Other</i>	Yes	No			
Joints	Normal	Yes	No	Erythematous	Yes	No
	Swelling	Yes	No	Reduced range of movement	Yes	No
	Tenderness	Yes	No	<i>Inability to weight-bear</i>	Yes	No
	<i>Other</i>	Yes	No			
Neonate	Normal	Yes	No	Umbilicus site clean	Yes	No
	Jaundice	Yes	No	Vaccination sites okay	Yes	No
	Skin rash	Yes	No			
	<i>Other</i>	Yes	No			

Prosthesis present at time of infection Yes No

	In-hospital peripheral intravenous cannula		In-hospital central intravenous cannula e.g. cut down
	Out-of-hospital peripheral intravenous cannula e.g. heparin lock		Out-of-hospital central intravenous cannula e.g. double lumen
	Intravascular graft or stent		Haemodialysis graft
	Prosthetic heart valve		Pacemaker/ defibrillator
	Joint replacement		Orthopaedic rod/ plate/ screws
	Peritoneal dialysis catheter		Other

Removable source of bacteraemia present Yes No

If present, was removable focus removed? Yes No

Date of removal ____/____/____

Date of repeat study culture / /

Number of cultures taken (starting with 1st culture positive for *S. aureus*): _____

If blood cultures positive, do they meet Duke major criteria? Yes No

If blood cultures positive, do they meet Duke major criteria?	Yes	No
If blood cultures positive, do they meet Duke minor criteria?	Yes	No

[illegible]

Community-acquired admission to hospital with an illness consistent with invasive *S. aureus* disease

Hospital-acquired positive *S. aureus* culture taken >48 hours after admission for another condition

Was surgery performed within previous 30 days of infection at the site of a previous surgery? Yes No

If yes, what type of surgery was performed?

If yes, when was the surgery performed? / /

1	Cutaneous abscess/ pustule	5	Open skin lesion	9	Surgery
2	Injection	6	Penetrating injury	10	Unknown
3	Intravenous device	7	Phlebotomy	11	Unknown, probable i.v. device
4	Other prosthetic material	8	Respiratory tract	12	Other

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Details of "other" or other "prosthetic material"																																																																																																			

Order		Type of infection	Order	Type of infection	
1	Abscess	9	Other skin lesion	17	Blood
2	Cellulitis	10	Diabetic foot infection	18	Contaminant
3	Furuncle/ pustule	11	Osteomyelitis – non vertebral	19	Empyema
4	Gangrene	12	Osteomyelitis – vertebral	20	Endocarditis
5	Infected burn	13	Pyomyositis	21	Mediastinitis
6	Skin ulcer	14	Septic arthritis	22	Meningitis
7	Wound – surgical	15	Peritonitis	23	Pneumonia
8	Wound – trauma	16	Pyelonephritis	24	Other

Type of abscess

If "other" or "other skin lesion" was chosen above, details:

Echo information

Has a TTE been done? Yes No If yes, when? ____ / ____ / ____

Height ____ cm Weight ____ kg

Defects seen	Mitral valve	Aortic valve	Tricuspid valve	Pulmonary valve
More than mild regurgitation				
Valve prolapse				
Thickened valve leaflet				
Prosthetic valve				
Vegetation				
Calcified valve				
Perforated valve				
Paravalvular abscess				
Ruptured chordae				

Other findings: _____

Duke Major echocardiographic criteria met? Yes No
Duke Minor echocardiographic criteria met? Yes No

Was vegetation seen by TTE? Yes No
Vegetation width (mm) ____ Vegetation length (mm) ____

Metastatic Sites of Infection

Metastatic infection is defined as a site of *S. aureus* infection resulting from spread from an initial site (via bloodstream seeding or direct extension).

Metastatic infection Yes (Y) No (N) Suspected (S)

Metastatic infection	Y/N/S	Culture	Imaging	Suspected from examination	Other details
Abscess					
Arthritis					
Epidural abscess					
Endocarditis					
Kidney abscess					
Meningitis*					
Vertebral osteomyelitis					

Non-vertebral osteomyelitis					
Psoas abscess					
Septic emboli					
Septic thrombophlebitis					
Other					

* Confirmed if positive CSF culture; suspected if raised WBC, high protein, low glucose

Follow-up

Follow-up date ___/___/___ How?

Details:

Outcome

	Outcome	Definitions
	Cure	Clinically improved, negative repeat culture, no additional sites of infection suspected or found
	Unresolved infection/ Treatment failure	Persistent fever, a new focus of infection that developed during treatment or persistent bacteraemia
	Death due to <i>S. aureus</i>	During admission or within 12 weeks of 1 st positive culture for <i>S. aureus</i>
	Death due to other causes	During admission or within 12 weeks of 1 st positive culture for <i>S. aureus</i>

If the outcome was death, when did the patient die? ___/___/___

Daily progress notes

Date, time	Notes	Signed
------------	-------	--------

Additional sepsis data
Date of 1st *S. aureus* culture: / / Study number

/ /	/ /	/ /
------	------	------

	AM	PM	AM	PM	AM	PM
Temp						
HR						
BP						
RR						

In: iv		
In: oral		
Out: urine		
Out: other		

☐ SIRS (2/4) Temp ≥38C or <36C HR >90 beats/min RR >20 breaths/min
WCC >12 x10³/µl or <4 x10³/µl or >10% immature band forms

FBC: WCC 10³/µl Band form % Hct % Hb g/dl
RBC transfusion: Yes/ No Volume transfused:

Renal failure: BUN (7-18 mg/dL) Cr (0.6-1.3 mg/dL) Date / /
 BUN (7-18 mg/dL) Cr (0.6-1.3 mg/dL) Date / /

Supplemental oxygen given: Yes/ No Nasal cannulae Mask Mask with bag
Ventilated: Yes/ No Bird Other ventilator:
O₂ monitoring: Yes/ No Sats only ABG

Septic shock: Yes/ No I/O record: Yes/ No Fluid bolus prescribed: Yes/ No
Cut down: Yes/ No Central line: Yes/ No CVP readings: Yes/ No Catheterised: Yes/ No
Vasopressors/ inotropes: Yes/ No Dose prescribed:

Cardiac arrest: Yes/ No

Coagulation: Plat 10³/µl PT: (10-14 sec) INR: APPT: (23-33 sec)

Liver failure: total bili (0-1 mg/dL) Alb (3.8-5.4 g/dL)

Diabetic: Yes/ No Blood sugar monitoring: Yes/ No Sliding scale: Yes/ No

Antibiotic therapy
MSSA
1st line: cloxacillin/ dicloxacillin/ approp cephalo (e.g. cefazolin, ceftriaxone, cefotaxime)
2nd line: augmentin/ clindamycin/ erythromycin/ imipenem/ sensitive Abx from testing
Not best practice: gent/ ceftaz/ amikacin/ cipro/ fuscidic/ septrin/ roxithro/ doxy/ cefoxitin

MRSA
1st line: vancomycin
2nd line: NA

Empiric: 1st/ 2nd/ Not Post-culture result: 1st/ 2nd/ Not

Polypharmacy: Yes/ No Max. no. of antibiotics simultaneously:

MRSA carriage study: Data collection sheets – Patients

Study number: _____ HN: _____ Ward: PICU 2 ICU surgery 2

Name: _____ Bed no. _____

Age: _____ years Sex: Male Female

Hospital admission date: ____/____/____; time _____ admitted from _____

ICU admission date: ____/____/____; time _____ admitted from _____

Reason for ICU admission: _____

ICU discharge date: ____/____/____; time _____ discharged to _____

Hospital discharge date: ____/____/____ Died: Yes/ No/ Discharged moribund
discharged to _____**Antibiotic therapy**

Antibiotic given	Start date	Stop date	Route	Dose and frequency	No. of days

Procedures

Date	On ward/ OR	Procedure

Investigations for infections

Date	Investigation	Result

Culture results (non-study)

Date	No. taken	Specimens taken	No. positive	Organism

Details on development of hospital-acquired infection (*S. aureus* or other):

Samples information

Study no. _____

	Admission	Discharge	Day 3	Day 7	Day 10	Day 14
Date						
Axilla (A)						
Result						
Tracheal (C)						
Result						
Nasal (N)						
Result						
2 swabs?						
Reason						
Throat (T)						
Result						
Urine (U)						
Result						
Wound (W)						
Result						
Site						
Wound						
Result						
Site						
Wound						
Result						
Site						

Results continued

Study no. _____

	Day ____	Day ____	Day ____	Day ____	Day ____	Day ____
Date						
Axilla (A)						
Result						
Tracheal (C)						
Result						
Nasal (N)						
Result						
2 swabs?						
Reason						
Throat (T)						
Result						
Urine (U)						
Result						
Wound (W)						
Result						
Site						
Wound						
Result						
Site						
Wound						
Result						
Site						

MRSA carriage study: Data collection sheets – Nursing staff

Study number: _____

Name: _____

Age: _____ years Sex: Male Female

Number of years working as a nurse: _____ years

Role: _____

Ward: PICU 2 ICU surgery 2

Samples information

Nasal swabs

	1 st screen	2 nd screen	3 rd screen
Date taken			
Swab number			
Culture result	MSSA MRSA Neither	MSSA MRSA Neither	MSSA MRSA Neither

Hand agar imprints

Dominant hand: Left Right

	1 st screen	2 nd screen	3 rd screen
Date taken			
Agar plate number			
Culture result	MSSA MRSA Neither	MSSA MRSA Neither	MSSA MRSA Neither